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Overanxious and underslept

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Are you feeling anxious? Did you sleep poorly last night? Sleep disruption is a recognized feature of all anxiety disorders. Here, we investigate the basic brain mechanisms underlying the anxiogenic impact of sleep loss. Additionally, we explore whether subtle, societally common reductions in sleep trigger elevated next-day anxiety. Finally, we examine what it is about sleep, physiologically, that provides such an overnight anxiety-reduction benefit. We demonstrate that the anxiogenic impact of sleep loss is linked to impaired medial prefrontal cortex activity and associated connectivity with extended limbic regions. In contrast, non-rapid eye movement (NREM) slow-wave oscillations offer an ameliorating, anxiolytic benefit on these brain networks following sleep. Of societal relevance, we establish that even modest night-to-night reductions in sleep across the population predict consequential day-to-day increases in anxiety. These findings help contribute to an emerging framework explaining the intimate link between sleep and anxiety and further highlight the prospect of non-rapid eye movement sleep as a therapeutic target for meaningfully reducing anxiety.

A lack of sleep amplifies anxiety in a dose–response manner[1,2]. Both chronic-partial as well acute-total sleep deprivation commonly and significantly increase anxiety in otherwise healthy individuals[2]. Further evidence for the anxiogenic impact of insufficient sleep comes from clinical science. Sleep disturbance is a recognized and common symptom of anxiety disorders[3]. Moreover, sleep disruption has been linked to the development and progression of anxiety disorders[4,5]—currently, the most common mental illness worldwide[6]. Testament to this link, sleep disturbance is present across the full axis of major anxiety disorders, including post-traumatic stress disorder (PTSD), generalized anxiety disorder, panic disorder and social anxiety disorder[3,7], suggesting trans-diagnostic applicability.

While the association between sleep loss and anxiety is well documented, here we address three key next-step questions. First, we investigate the underlying neural basis of why and how a lack of sleep amplifies anxiety in humans. Second, we determine whether specific features of sleep (stages and physiology) beneficially prevent the escalation of anxiety associated with insufficient sleep, therefore acting as an anxiolytic. Third, we examine whether subtle, societally common sleep deficits within an individual, from one night to the next, trigger consequential day-to-day increases in anxiety.

Concerning the first question, and independent of sleep, a recognized network of brain regions is associated with heightened anxiety in healthy adults[8,9] and shows marked alterations in clinical anxiety disorders[10]. These regions include the dorsal anterior cingulate (dACC)[11,12] and the amygdala[13–15], associated with greater reactivity to negative emotions[8,10,11]. Similar hypersensitivity has been observed in the insula, potentially reflecting increased aversive anticipation signalling in anxiety[9,16].

In marked contrast, hypoactivity within the medial prefrontal cortex (mPFC) has been reported in high-trait-anxious individuals as well as in patients diagnosed with anxiety disorders[11,12,17], typically accompanied by impaired functional coupling of the mPFC with the amygdala at rest[18,19]. These prefrontal impairments may reflect a deficit in emotional control, manifested in increased anxiety temperament[20,21] or, clinically, in generalized anxiety[10].

Pertaining to the second question of the anxiolytic benefit of sleep, patients suffering from anxiety disorders express reductions in non-rapid eye movement (NREM) sleep. This includes reductions in the amount of NREM slow-wave sleep, reported in generalized anxiety disorder[22–24], panic disorders[25], patients with PTSD[26] and in healthy individuals with high-trait anxiety[27], accompanied by corresponding changes in electroencephalogram (EEG) slow-wave activity (SWA, 0.5–4.0 Hz)[28,29]. Relevant to the third question, these sleep-stage changes also co-occur with reductions in subjective sleep quality[3] as well as objective sleep efficiency[7,22,23,25], evident in clinical[23] and healthy[30] cohorts. Such cross-sectional (between subjects) evidence demonstrates that poor sleep quality positively correlates with higher anxiety.

Building on this evidence, here we seek to test three inter-related hypotheses targeting these unanswered questions: (1) that the underlying neural mechanism explaining the anxiogenic impact of sleep loss involves hypoactivity and associated reduced functional connectivity of the mPFC, yet increased activity within the limbic amygdala and associated insula, the extent of which will linearly scale with the degree of amplified anxiety across individuals; and conversely (2) that NREM sleep, and specifically NREM SWA, supports a palliative anxiolytic benefit through regulation of the above neural networks, thereby preventing the escalation of anxiety that would otherwise occur following continued wakefulness; and (3) using a micro-longitudinal study, that changes in sleep quality within an individual, from one night to the next, result in consequential day-to-day changes in anxiety, such that a worsening in nightly sleep quality would lead to a relative increase in anxiety the next day.

In short (but see Methods), in the first series of studies, two independent samples were recruited: (1) 18 healthy participants took part in an in-laboratory experimental study and (2) a subsample of the general population ($n = 194$) took part in an online micro-longitudinal study. The in-laboratory experimental study required participants to take part in two separate sessions: one after a full night of sleep (a sleep-rested (SR) session) and a second following 24 h of wakefulness (a sleep-deprived (SD) session). In each session, participants rated their level of anxiety in the evening and morning at the same circadian times (see Fig. 1a). This was followed by a functional magnetic resonance imaging (fMRI) scan using an affective assay paradigm—averse emotional clips—known to provide a

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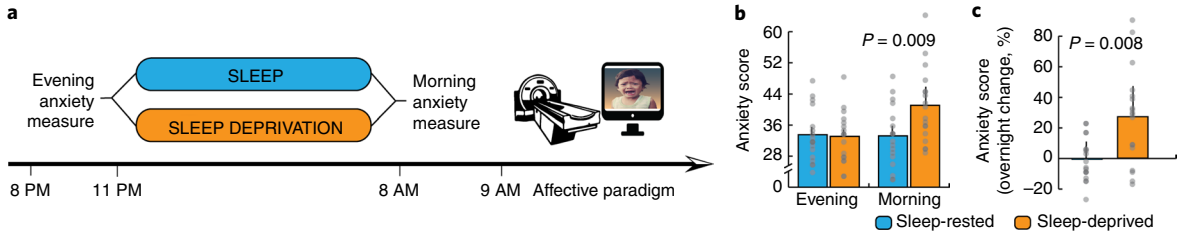

Fig. 1 | Experimental design and behavioural results. a, In-laboratory experimental design: a repeated-measures counterbalanced protocol across 18 participants. Anxiety levels were measured before and after a sleep manipulation protocol, followed by a functional MRI scan that included an affective paradigm (viewing of emotional and neutral clips). **b**, Anxiety levels were similar in the evening of each session, before any sleep manipulation (left, mean difference = 0.44 ± 1.08, 95% CI = [−1.84, 2.7], $t(17) = 0.41$, $P = 0.6$), yet were significantly greater following SD relative to SR (right, mean difference = 7.83 ± 2.65, 95% CI = [−13.43, −2.22], $t(17) = 2.95$, $P = 0.009$). **c**, Overnight changes in anxiety levels revealed a 30% increase in anxiety following SD, yet no change following the SR night (sleep × time interaction, $F(1,16) = 8.95$, $\eta^2 = 0.34$, $P = 0.008$). Error bars denote s.e.m. Individual data points are marked in grey.

measure of emotional brain function[31].The online micro-longitudinal study tracked the habitual sleep and subjective anxiety levels of sample participants across two consecutive nights/days. Measures of sleep quality and efficiency were assessed for each participant in each study night.

A second series of confirmation studies were subsequently conducted to replicate and extend key findings. Here two additional independent samples were recruited: (1) 32 healthy participants took part in an in-laboratory overnight sleep study to examine replication of the association of NREM sleep with anxiety in an independent dataset, and (2) a subsample of the general population ($n = 154$) took part in a second online microlongitudinal study, now tracking their habitual sleep and subjective anxiety across a longer duration of four consecutive nights/days to replicate and further define the directionality of the sleep–anxiety association.

Results

In-laboratory study: sleep-loss-induced anxiety. Anxiety scores were analysed using a repeated measures analysis of variance (ANOVA), with the factors sleep (SD, SR) and time (morning/evening). Fitting with the anxiogenic experimental hypothesis, SD was associated with significantly higher anxiety scores (sleep main effect, $F(1,17) = 6.19$, mean difference = 3.69 ± 1.48, $\eta^2 = 0.27$, 95% confidence interval (CI) = [0.56, 6.82], $P = 0.02$, Bonferroni corrected; Fig. 1).

Critically, this effect was evident only in the morning following sleep manipulation (main effect of time, $F(1,17) = 12.02$, mean difference = 3.86 ± 1.11, $\eta^2 = 0.41$, 95% CI = [1.51, 6.21], $P = 0.003$, Bonferroni corrected), further reflected by a significant interaction between sleep and time ($F(1,16) = 8.95$, $\eta^2 = 0.34$, $P = 0.008$; Fig. 1c). Specifically, on the evening of each experimental night and before either of the experimental manipulations, participants started each session with statistically equivalent levels of anxiety (SR = 33.50 ± 1.61, SD = 33.06 ± 1.58, mean difference = 0.44 ± 1.08, 95% CI = [−1.84, −2.73], $t(17) = 0.41$, $P = 0.67$; Fig. 1b). However, the following morning, there were significant differences. SD resulted in a 30% increase in anxiety relative to the SR condition (SR = 33.22 ± 1.86, SD = 41.06 ± 2.27, mean difference = 7.83 ± 2.65, 95% CI = [2.22, 13.43], $t(17) = 2.95$, $P = 0.009$; Fig. 1b).

Notably, 78% of all participants in the SD condition reported an increase in anxiety, confirming a robust impact of sleep loss on the escalation of anxiety in healthy individuals. Of clinical relevance, 50% of all participants exceeded anxiety scores >40 following SD—a cut-off typically used to determine the presence of clinical symptoms of anxiety[32].

In-laboratory study: neural correlates of sleep-loss-induced anxiety. Functional MRI analyses focused a priori on anxiety-sensitive brain regions of interest: the amygdala, dACC, insula and mPFC; see Supplementary Table 1). First, and independent of any association with anxiety, three of the four regions of interest (ROIs) expressed a significant interaction of sleep and emotion—the mPFC, amygdala and dACC (Fig. 2).

Second, consistent with findings in anxiety disorders[33,34], the mPFC expressed significant hypoactivity following SD, relative to the SR condition, in response to emotional stimuli (interaction of sleep and emotion, $F(1,17) = 4.88$, $\eta^2 = 0.22$, $P = 0.04$; sleep means for emotional versus neutral activation, SR = 0.16 ± 0.06, SD = −0.04 ± 0.03, mean difference −0.2 ± 0.09, 95% CI = [−0.39, 0]; Fig. 2a). The opposite was true for the amygdala and dACC, both of which demonstrated amplified activity in the SD condition for emotional content (bilateral amygdala interaction of sleep and emotion, $F(1,17) = 6.09$, $\eta^2 = 0.26$, $P = 0.02$; sleep means, SR = 0.07 ± 0.04, SD = 0.29 ± 0.06, mean difference = 0.21 ± 0.08, 95% CI = [−0.4, −0.03], with stronger left-sided effects for the amygdala. dACC interaction $F(1,17) = 6.04$, $\eta^2 = 0.26$, $P = 0.02$; sleep means, SR = −0.09 ± 0.03, SD = 0.04 ± 0.04; mean difference = 0.13 ± 0.05, 95% CI = [0.01, 0.24]; Fig. 2b,c). No interaction of sleep loss and emotion was observed in the insula (sleep means, SR = −0.12 ± 0.04, SD = −0.1 ± 0.04; mean difference −0.01 ± 0.07, 95% CI = [−0.16, −0.13], $t(17) = -0.15$, $P = 0.8$; Bayesian analysis in support of the null hypothesis was substantial: $BF_{01} = 4.07$, 95% CI = [−0.4, 0.45]); Fig. 2d).

Combined, these results demonstrate increased emotional reactivity following sleep loss within the extended limbic network of the amygdala and dACC, together with a loss of typical emotional regulation involvement of the mPFC—a neural signature matching that reported in meta-analyses of anxiety disorders, as well as in high-anxiety individuals[10,11,17].

Having established main effects of sleep loss on brain activity in specific a priori ROIs, we next tested our first core hypothesis: that across individuals, sleep loss-related changes in brain activity significantly predicted the extent of amplified anxiety caused by sleep deprivation.

Supporting the experimental hypothesis, and again fitting a prototypical neural signature of anxiety, the extent of hypoactivity in mPFC following sleep deprivation predicted the increase in anxiety caused by sleep loss (Fig. 3a; $R = -0.58$, 95% CI = [−0.15, −0.82], $P = 0.01$): the greater the impairment in mPFC activity following sleep deprivation, the larger the sleep-loss-induced increase in anxiety. Importantly, these sleep loss-associated changes with anxiety remained significant when controlling for corresponding changes in mood states ($P < 0.05$; see Supplementary Note 1a). Therefore, the sleep-loss changes in mPFC activity were accounted for by changes in anxiety, above and beyond changes in mood.

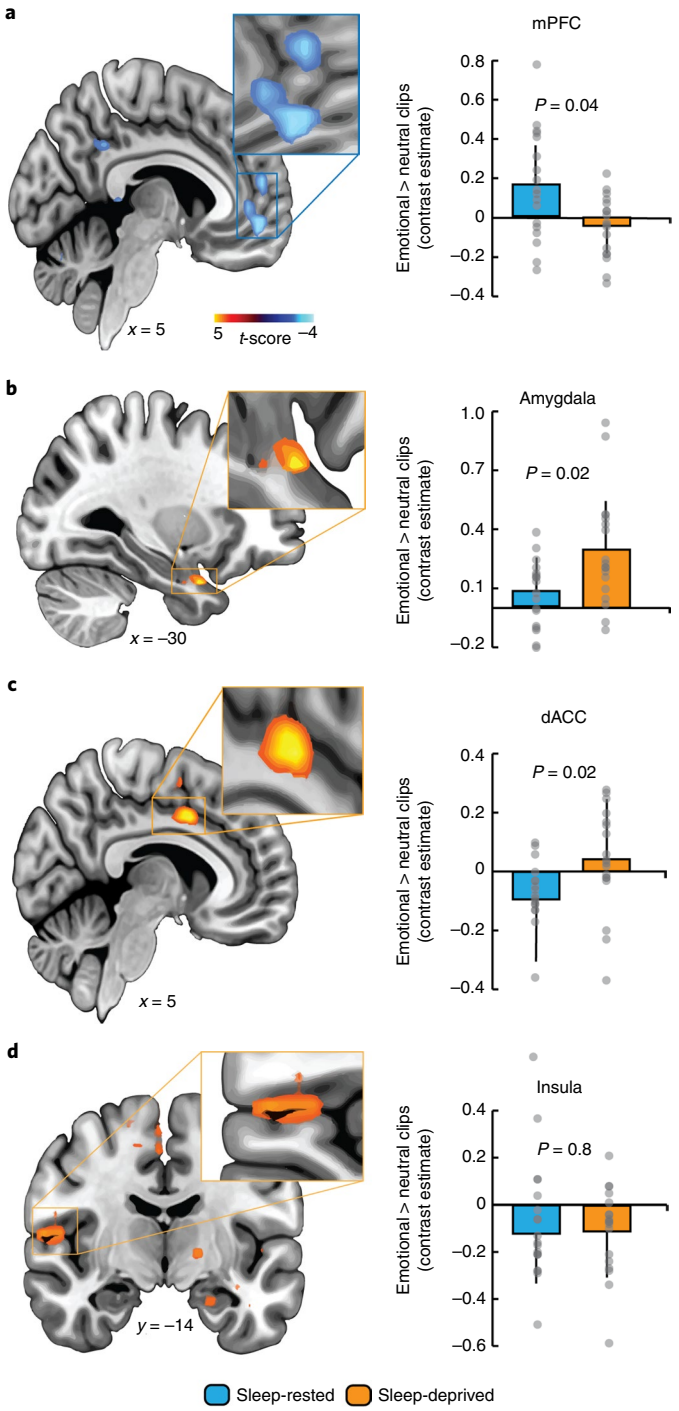

Fig. 2 | fMRI results of the In-laboratory study. a–d, Significant clusters of activation (left) from an exploratory whole-brain analysis during the viewing of emotional relative to neutral clips, showing decreases in the emotion-regulation regions of the mPFC (**a**) following SD (blue). In contrast, sleep-loss-related increases in activity were observed in the extended limbic network including the amygdala (**b**), dACC (**c**) and insula (**d**) (orange; presented for illustration purposes only at $P<0.005$; Montreal Neurological Institute (MNI) coordinates are denoted for each slice; see Supplementary Table 3). Right: ROI analysis within a priori regions in the extended limbic network demonstrating that SD triggered a decrease in mPFC activity in response to emotional relative to neutral content ($F(1,17)=4.88$, $\eta^2=0.22$, $P=0.04$) while elevating emotional-related activity in both limbic-associated regions of the dACC ($F(1,17)=6.04$, $\eta^2=0.26$, $P=0.02$) and the amygdala (averaged for bilateral amygdala, $F(1,17)=6.09$, $\eta^2=0.26$, $P=0.02$), without a significant difference in insula activity (averaged for bilateral insula, $t(17)=-0.15$, $P=0.8$, $BF_{01}=4.07$). Error bars denote s.e.m. Individual data points are marked in grey.

No such relationships with anxiety were observed with activity in the other a priori ROIs showing main effects of sleep loss: the amygdala or dACC (all $R<0.2$, $P>0.2$; Bayesian analysis in support of the null hypothesis was substantial: amygdala, $BF_{01}=3.43$, 95% CI = [0.43, −0.43]; dACC, $BF_{01}=3.07$, 95% CI = [−0.33, 0.52]). Such findings suggest a selective associational relationship between mPFC hypoactivity and the anxiogenic impact of sleep loss, one that may be due more to the loss of emotional control within the mPFC[11,12,17], rather than in more rudimentary emotional-reactivity regions such as the amygdala[13,14].

We next sought to test the hypothesis that sleep loss-related impairments in mPFC connectivity, beyond changes in mPFC activity, further accounted for the sleep-loss induced increase in anxiety levels. Here we specifically focused a priori on functional connectivity between mPFC–amygdala due to its established link with high-anxiety states and anxiety disorders[18,35–37].

Consistent with the profile observed in clinical anxiety cohorts, SD resulted in a significant impairment in mPFC–amygdala connectivity relative to the SR condition (main effect of sleep in bilateral amygdala, $F(1,17)=5.04$, mean difference $=-0.16\pm0.07$, $\eta^2=0.23$, 95% CI = [−0.3, −0.1], $P=0.04$, Bonferroni corrected; Fig. 3b).

However, this main effect of sleep deprivation on amygdala connectivity with this specific region of the mPFC was not predictive of inter-individual differences in anxiety following sleep deprivation ($R=0.27$, $P=0.3$; Bayesian analysis in support of the null hypothesis was weak: $BF_{01}=1.91$, 95% CI = [0.623, −0.203]). The lack of a significant association may be due to subtly different regions of mPFC being more predictive of inter-individual differences in anxiety levels, beyond main effects of anxiety[10,38]. We therefore examined, post hoc, whether our secondary ROIs demonstrated sensitivity. This was the case, wherein amygdala connectivity with an mPFC region 4 mm dorsal to our primary prefrontal ROI ($x,y,z(12,62,20)$) significantly predicted the extent of inter-individual differences in anxiety following sleep deprivation ($R=-0.46$, 95% CI = [0, −0.76], $P=0.05$, with stronger effects for left amygdala connectivity). This result is indicative of impaired top-down regulatory control proposed in neural models of anxiety[18,19,35–37], and it suggests that greater impairment in mPFC–amygdala connectivity is associated with a larger increase in anxiety following sleep deprivation.

Together, these findings demonstrate that the main experimental effect of amplified anxiety following sleep loss is associated with main-effect increases in emotion-generation regions of the amygdala and dACC, yet hypoactivity of the mPFC and impaired mPFC–amygdala connectivity. Moreover, the magnitude of mPFC impairment specifically and selectively predicted inter-individual differences in anxiety caused by a lack of sleep, beyond main effects.

In-laboratory study: sleep physiology in relation to anxiety and emotional brain dynamics. The second experimental hypothesis investigated a converse question: what is it about the presence of sleep, rather than its absence, that provides a palliative anxiolytic function? Specifically, we tested the hypothesis that indices of NREM slow-wave sleep (SWS) and associated spectral SWA prevent the escalation of anxiety associated with continued wakefulness, as observed in the SD condition.

Consistent with the prediction, NREM SWS predicted the degree of overnight reduction in anxiety from evening to morning (within-subject measure, $R=-0.52$, 95% CI = [−0.07,−0.79], $P=0.03$) in the SR condition and predicted the outright next-morning anxiety score ($R=-0.59$, 95% CI = [−0.15, −0.8], $P=0.01$; Fig. 4a). That is, those individuals with greater SWS expressed a greater evening-to-morning dissipation of anxiety and thus lower next-day anxiety levels.

Importantly, the association between NREM SWS and anxiety was specific to state anxiety, with no significant association with trait anxiety scores ($R=-0.17$, $P=0.5$; $BF_{01}=2.76$, 95% CI [0.29,−0.55]).

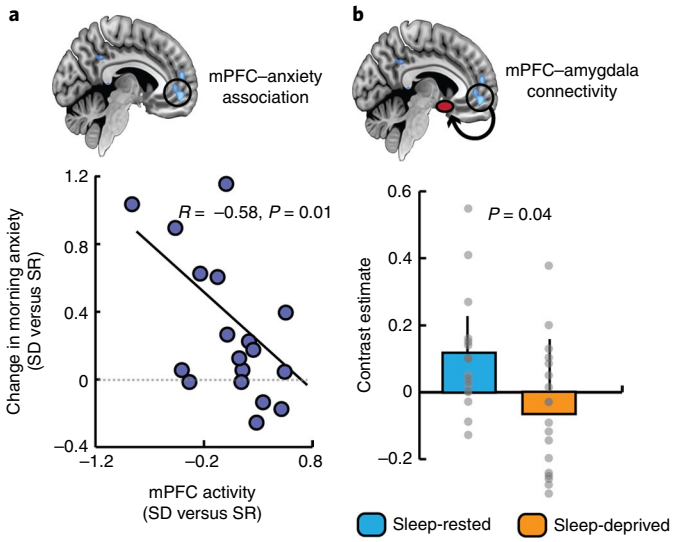

Fig. 3 | mPFC activity in relation to anxiety. a, Significant decreases in mPFC activity were associated with higher anxiety levels in the SD state relative to SR ($R=-0.58$, 95% CI = [−0.15, −0.82], $P=0.01$). **b**, mPFC–amygdala connectivity (calculated within a priori ROIs) was reduced following sleep deprivation relative to the rested condition (main effect of sleep across left and right amygdala, $F(1,17)=5.04$, mean difference = −0.16 ± 0.07, $\eta^2=0.23$, 95% CI = [−0.3,−0.1], $P=0.04$). Error bars denote s.e.m. Dashed grey line denotes zero crossing. Individual data points are marked in grey.

Thus the relationship between NREM SWS and anxiety appears to be preferentially linked to the regulation of daily fluctuations in anxiety state, separate from a more stable anxiety trait.

In addition to NREM SWS quantity, NREM spectral EEG quality, specifically SWA (0.5–4.0 Hz), demonstrated a similar relationship. Here, greater amounts of NREM SWA, especially in posterior topographical regions, predicted a greater overnight reduction in anxiety from evening to morning across individuals ($R=-0.62$, 95% CI = [−0.2, −0.84], $P=0.007$) and lower absolute anxiety scores the next day (Fig. 4b; $R=-0.53$, 95% CI = [−0.06, −0.8], $P=0.03$; both calculated for posterior channel derivations). Similar to SWS duration, the association of NREM SWA with anxiety was specific to changes in state anxiety, and not significantly related to trait anxiety ($R=0.005$, $P=0.9$; Bayesian analysis in support of the null hypothesis was substantial: $BF_{01}=3.33$, 95% CI = [0.44,−0.44]). Moreover, the association of NREM SWA with next-day anxiety remained significant when controlling for co-occurring changes in mood ($P<0.05$; see Supplementary Note 1a), confirming a selective anxiolytic benefit of SWA beyond co-morbid mood fluctuations. Together, these findings support the hypothesized palliative potential of NREM SWS—both quantity and electrophysiological quality—in preventing the escalation of state anxiety that otherwise develops in the absence of sleep.

Last, we tested the hypothesis that the overnight regulation of anxiety by NREM sleep across the SR condition was further associated with neural changes in affective brain activity observed the next morning. Specifically, analyses focused on the brain region showing sleep-dependent sensitivity to anxiety in the rested relative to deprived condition—the mPFC.

Following the SR night, greater next-day mPFC (re)engagement across individuals was associated with significantly lower next-day anxiety ($R=-0.59$, 95% CI = [−0.16, −0.84], $P=0.01$; Fig. 4c). Once again, this sleep-dependent association was unique to state levels of anxiety, with no significant associations observed for stable anxiety traits ($R=-0.009$, $P=0.9$; $BF_{01}=3.21$, 95% CI = [0.35,−0.5]). Furthermore, time spent in both NREM SWS and NREM SWA spectral activity (in posterior channel derivations) predicted greater mPFC re-engagement the next day (NREM SWS, $R=0.5$, 95% CI = [0.03, 0.79], $P=0.04$; NREM SWA, $R=0.55$, 95% CI = [0.09, 0.81], $P=0.02$; Fig. 4c). These results support an anxiolytic role for NREM sleep by restoring prefrontal mechanisms known to be critical for the regulation of anxiety[10,20,21].

A second independent polysomnography (PSG) study ($n=32$) was conducted to replicate these findings, but with the difference that sleep was recorded in the laboratory, rather than at home as in the first study. These data replicated the original findings (see Extended Data Fig. 1 and Supplementary Note 2) and establish that sleep-recording context (home/in-lab) did not change the above anxiety associations.

Online micro-longitudinal studies 1 and 2: night-to-night perturbations in sleep quality negatively impact next-day anxiety. The in-laboratory experiment established that acute total sleep deprivation triggers an anxiogenic impact, one that is associated with an underlying change in the affective network of prefrontal- and limbic-associated regions. Our final experimental hypothesis, however, sought to determine whether ecologically modest night-to-night variations in sleep, within an individual, were associated with consequential day-to-day changes in subjective anxiety. In online study 1, habitual sleep and next-day subjective anxiety were tracked for two consecutive days within the same individuals, and in online study 2 the longitudinal window was extended to four consecutive days.

Supporting the experimental prediction, and the directional predictions from the in-laboratory study, there was a significant association between night-to-night changes in sleep efficiency and subsequent day-to-day changes in experienced anxiety: individuals who experienced a reduction in sleep efficiency from one night to the next reported a corresponding and significant increase in next-day anxiety, and vice versa ($F(1,93)=4.14$, mean change = 0.3 ± 0.46 and −0.98 ± 0.39, respectively, $\eta^2=0.043$, $P=0.04$). In addition to this categorical approach, we further tested the hypothesis using a correlational (continuous variable) approach between sleep efficiency and anxiety (see Methods). Here again, the greater the night-to-night change in sleep efficiency (increase versus decrease), the greater the consequential change in anxiety state the following day (decrease versus increase, respectively, $R=-0.15$, $P=0.036$, $n=194$). Similar findings were observed in online study 2, where sleep and subjective anxiety were tracked for an extended duration of 4 d. Individuals in this study reported significantly greater subjective anxiety the following day if they slept worse than they usually did, relative to days when they slept better than usual ($\beta=-14.77$, 95% CI = [−20.4, −9.08], $t(42.06)=-5.27$, $P<0.0001$, $R^2=0.053$).

Notably, these effects were not evident when examining changes in sleep duration (all $P>0.5$; see Supplementary Note 3), indicating that measures of sleep continuity serve as better predictors of subsequent anxiety than sleep duration alone. As with sleep efficiency, night-to-night changes in subjective sleep quality similarly predicted changes in next-day anxiety in a bidirectional manner (all $P\leq0.001$ see Supplementary Note 3).

Although these findings describe a clear association between sleep and next-day anxiety, they leave open the question of the directional influence of this association. The link between anxiety and poor sleep is often bidirectional, such that levels of bedtime anxiety can lead to worse sleep and, in turn, worse sleep can lead to greater level of anxiety the next day[39,40]. To estimate the unique contribution of sleep to next-day anxiety, we conducted two complementary analyses in each online study (see Supplementary Note 4 for more details). In online study 1, we examined the association between sleep and anxiety on the second day of the study while controlling for anxiety levels present on the first day (that is, before

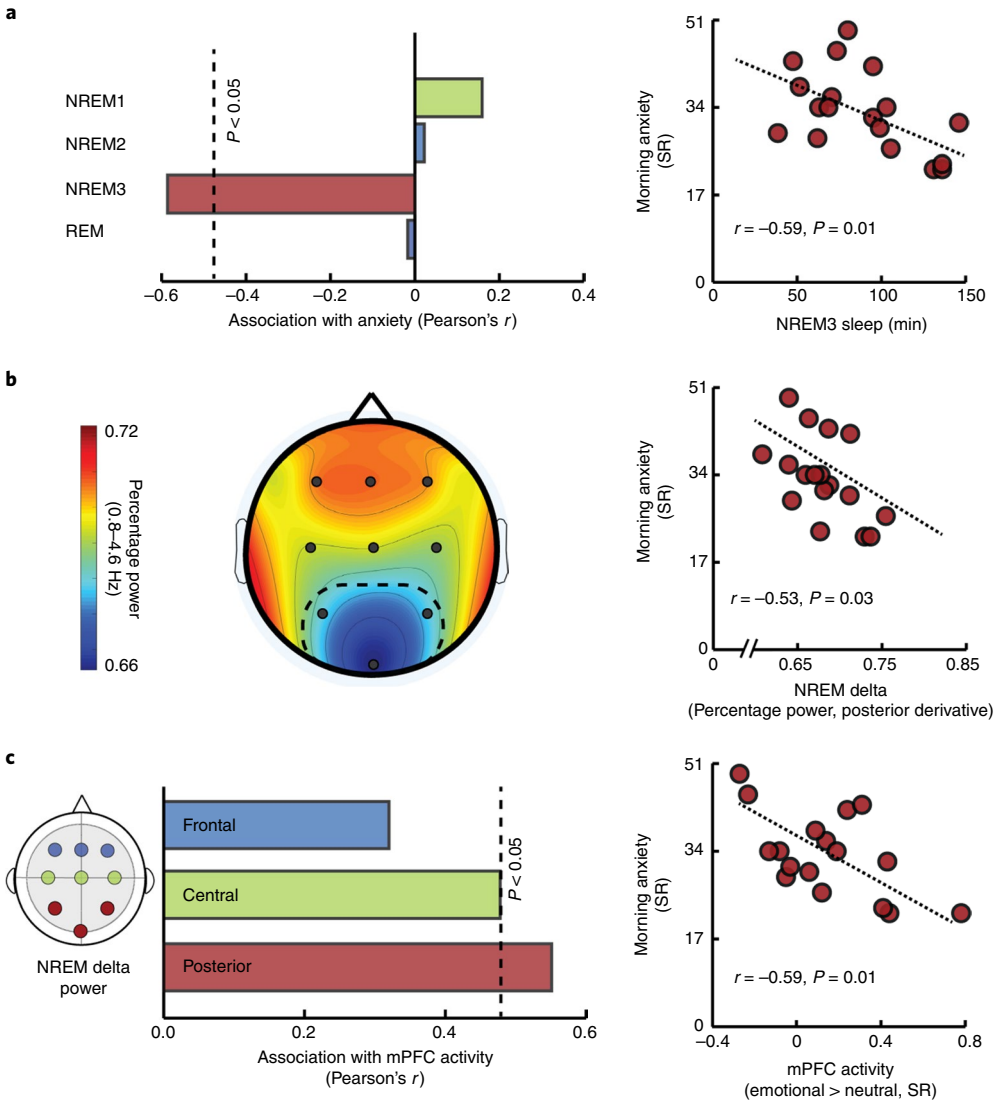

Fig. 4 | Sleep-rested physiology in relation to next-day anxiety. a, Anxiety association in relation to REM and NREM sleep stages (left). Only time spent in deep NREM sleep (NREM3) was associated with a significant reduction in next-day anxiety (right). **b**, Power in the delta band (SWA, 0.8–4.6 Hz) during NREM sleep (left) was associated with lower morning anxiety (right), most pronounced for posterior derivations (dashed circle). **c**, Greater mPFC activity in the SR session was associated with higher SWA during NREM sleep (left, $R = 0.55$, $P = 0.02$ calculated for posterior derivations) and a significant decrease in next-day morning anxiety across participants (right).

sleep took place). In online study 2, we conducted a lag analysis where the association between sleep efficiency and subjective anxiety was controlled for by the level of anxiety the previous day (see Methods). These analyses revealed that the association between sleep efficiency and anxiety is evident even when controlling for previous anxiety states (online study 1, $R = -0.2$, $P = 0.005$; online study 2, $\beta = -16.94$, $t(48.37) = -4.83$, $P < 0.0001$).

Anxiety is often co-morbid with alterations in mood[41], and both have been associated with poor sleep[42,43]. We therefore examined the impact of mood states on the association of habitual sleep and anxiety in our online studies. These control analyses reveal that sleep efficiency still significantly predicted anxiety levels across participants in both online studies, when controlling for co-variations in mood ($P = 0.002$–0.004; see Supplementary Note 1b). In addition to these control analyses, we added a secondary anxiety assessment to online study 2—the Beck Anxiety Inventory (BAI[44]; see Methods), as this takes into account somatic measures of anxiety and is less collinear with mood states[45]. Similar to the findings using the state-trait anxiety inventory (STAI) measure, analyses of BAI data revealed that worse sleep efficiency was associated with greater subjective anxiety the following day ($\beta = -13.49$, 95% CI = [−21.32, −5.65], $t(61.27) = -3.44$, $P = 0.001$; see Supplementary Note 1b).

In sum, ecologically relevant night-to-night variability in sleep within individuals predicted consequential day-to-day changes in subjective anxiety state—an effect that was independent of alterations in mood as well as in trait anxiety. These micro-longitudinal experiments not only corroborate the results of the in-laboratory experiment manipulation of acute total sleep deprivation, but demonstrate that subtle alterations in nightly sleep quality are sufficient to result in consequential next-day changes in anxiety.

Discussion

Taken together, these findings set forth a mechanistic neural framework explaining how and why insufficient sleep may contribute to anxiety and, conversely, establish a palliative function of NREM SWS capable of ameliorating anxiety. Moreover, these effects were demonstrable across both acute experimental sleep deprivation and following modest night-to-night changes in sleep quality.

Prior meta-analyses have described a robust anxiogenic impact of sleep loss[2]. Importantly, these effects are evident across a multitude of study contexts[46,47], and when sleep is restricted to only 3 h per night relative to a whole night of sleep[48]. Consistent with these data, we establish that one night of sleep deprivation triggered a significant increase in anxiety in otherwise healthy participants. Indeed, following sleep deprivation, 50% of our experimental participants expressed levels of anxiety that exceeded the clinical threshold described in typical anxiety disorders[32]. Such findings affirm the strong co-morbidity of sleep disruption and anxiety, wherein chronic sleep disruption more than doubles the risk of developing an anxiety disorder[4,5]. Our experimental studies demonstrate that, within this interaction, sleep loss can causally and directionally instigate high levels of anxiety in individuals who were otherwise non-clinically anxious when sleep-rested. This finding defines a causal influence of disrupted sleep on the development of anxiety, beyond simply a co-occurring symptom of anxiety disorders[49] (though the inverse is similarly true, indicating a bidirectional model of causal interaction[39,40]).

That anxiety and sleep disruption are co-morbid and causally interactive suggests that they may converge by way of a common underlying neural mechanism[50]. The current study addressed this question, focusing on a well-characterized network associated with the condition of anxiety—the amygdala, dACC, insula and mPFC. Though sleep deprivation effects were observed in the amygdala, dACC (both hyperactivity) and mPFC (hypoactivity), the interaction between sleep deprivation and the magnitude of anxiety increase was specifically accounted for by changes in mPFC activity and associated mPFC connectivity.

Impaired mPFC activity is a recognized neural phenotype of highly anxious individuals, both in clinical and non-clinical populations[11,12,17,18]. Similarly, mPFC hypoactivity predicts worse symptom severity in the anxiety disorder of PTSD[51]. In addition to mPFC hypoactivity, reduced mPFC–amygdala coupling—an index of impaired prefrontal control of limbic activity—correlates with anxiety levels across healthy individuals[18] and in those with generalized anxiety disorder[52], social anxiety[19] and PTSD[51].

Our findings establish that one night of sleep loss in otherwise healthy individuals triggers an anxiogenic neural profile that is prototypical of anxiety disorder—decreases in mPFC activity and limbic connectivity alongside hyperactivity in amygdala and dACC[10,38]. Most relevant, we show that the degree of mPFC disengagement, rather than changes in other affect-related regions, expressly predicted the magnitude of anxiety increase across individuals caused by sleep loss.

Regions of the anterior mPFC play a critical role in adaptive emotional processing, including the discernment of emotional stimulus significance as well as top-down regulation of corresponding affective responses that, if impaired, can lead to biased threat perception[33,34]. This is especially true for the ventral subregions of the mPFC that are anatomically connected to core emotion-processing regions such as the amygdala[18], further linked to excess peripheral endocrine and autonomic responses to stress[53]. Notably, insufficient sleep robustly impairs mPFC activity and associated limbic functional connectivity[42,54,55].

Our data help define an emerging neuropathological model in which sleep disruption contributes to the maintenance and/or exacerbation of anxiety through impaired mPFC engagement. The manifold consequences may include loss of adequate emotion regulation and excess emotional responsivity, together with maladaptive states of autonomic hyperarousal, including sympathoadrenal and hypothalamic–pituitary–adrenal overactivity.

Our second core experimental question examined what it is about sleep, physiologically, that provides an overnight, anxiolytic benefit. Across the SR night in both in-laboratory studies, greater NREM SWA (and associated SWS) predicted a greater overnight reduction in anxiety the next day. Moreover, the same measure of NREM SWA and SWS additionally predicted the degree of next-day re-engagement of mPFC activity, post-sleep.

Functionally, NREM SWA has previously been associated with hippocampal memory processing[56]. Our findings suggest a functional role for NREM SWA—an anxiolytic brain benefit preventing the overnight escalation of anxiety. This role of NREM sleep can be considered separable from the functional role of REM sleep in the regulation of emotions[42] based on the temporal mechanisms underlying each. Specifically, anxiety (NREM associated) is in the domain of mood states that operate across a time frame of hours[57], while emotional reactivity (REM associated) is considered to be a short-term, acute process that begins and ends within a time frame of milliseconds to minutes[57].

Further support for the proposed anxiolytic role of NREM sleep comes from clinical studies that have described impairments of NREM SWS in patients suffering from anxiety disorders[7,25]. Disrupted NREM SWS has been reported in generalized anxiety disorder[22–24], panic disorders[25] and PTSD[26], intimating that the relationship of, and signature impairment in, NREM SWS is transdiagnostic and common across these anxiety categories. At the subclinical level, individuals with high-trait anxiety express lower NREM SWS relative to low-trait anxiety individuals[27]. Even mild anticipatory anxiety and apprehension regarding next-day events reduces NREM SWS[58]. Combined with our current results, this collection of evidence indicates a sensitivity of NREM SWS and associated SWA to numerous forms of anxiety, an issue discussed further in the findings of the micro-longitudinal online study, below.

Focusing on this sleep-dependent anxiolytic mechanism, reductions in NREM SWA have been observed in primary insomnia[59] and further associated with aberrantly high sympathetic autonomic activity[60]. Conversely, NREM SWS reflects a homoeostatic state of marked parasympathetic dominance[61], coupled with a reduction in central autonomic network activity within the brain. In this context, posterior cingulate regions and the precuneus regulate parasympathetic activity associated with lowered anxiety[62,63] while anterior midline regions, including both the ACC and the amygdala, have been shown to regulate sympathetic activity[64] linked to high anxiety.

Building on this evidence, we propose a restorative, autonomic-related function of SWS on high-order affective brain networks, especially those associated with autonomic control[62]. Herein, adequate NREM SWA proffers an anxiolytic benefit through next-day restoration of cingulate regions relevant to parasympathetic regulation. This, in turn, downregulates mPFC-related limbic functioning, thereby optimizing both affective regulation within the brain and associated peripheral sympathovagal responses within the body that lower anxiety states[11,17,18,38]. Notably, the strength of NREM SWA association with reduced next-day anxiety was most pronounced over posterior derivations. Such a gradient asserts the importance of posterior midline brain regions involved in parasympathetic regulation, aiding in the overnight restoration of autonomic balance that occurs during SWS[65]. Moreover, NREM SWS suppresses activity of the hypothalamic–pituitary–adrenal axis[66], when cortisol levels consequently decline[67], complementing a state of parasympathetic dominance. Conversely, this framework would suggest that reductions in NREM SWS, and/or impairment of NREM SWA within an individual may serve as a biomarker of amplified autonomic nocturnal arousal and thus anxiety, especially considering the central role of sympathetic hyperactivity in neurobiological explanations of anxiety disorders[68,69].

Our final experimental question addressed whether modest nightly reductions in sleep quality from one night to the next, within individuals, would result in a corresponding increase in anxiety from one day to the next.

Consistent with the anxiogenic impact of sleep deprivation, we establish that even subtle perturbations in sleep quality from one

night to the next negatively impact anxiety—worse night-to-night sleep quality (efficiency) predicted consequential elevation in anxiety the following day. These findings build on clinical and cross-sectional data that reported associations between lower sleep efficiency in patients with anxiety disorders relative to controls[23] and, in a more general population sample, that self-reported poor sleep quality positively correlates with higher anxiety[30].

Of note, co-variate analyses demonstrated that the relationship between sleep loss and anxiety (both at the neural and behavioural level) remained significant, even when taking into account co-occurring changes in mood (see Supplementary Note 1). Moreover, this was true when using an alternative assessment of anxiety known to be less collinear with mood (BAI[44]; see Methods).

Though the online micro-longitudinal study was not designed to explore the direction in which disturbed sleep and anxiety interact, our findings point towards impaired sleep as a strong modulator of next-day anxiety even when statistically controlling for previous anxiety states (see Supplementary Note 4). This does not invalidate the bidirectional nature of the interaction between sleep and anxiety, with several reports demonstrating that daytime anxiety can be associated with subsequent impairments in sleep quality[39,40]. Indeed, such bidirectionality has the potential for creating a negative feedback cycle wherein sleep disruption and escalating anxiety become self-reinforcing[39], offering mechanisms contributing to the initial instigation of anxiety disorders as well as their ongoing maintenance and/or worsening[4,5].

That even modest reductions in sleep quality impact next-day anxiety is relevant given the continued erosion of sleep time in developed nations (National Sleep Foundation Sleep in America Poll, 2013), and the high prevalence and economic health burden of anxiety disorders in these same countries[6]. Considering that 70–80% of patients suffering from anxiety disorders report restless and unsatisfying sleep[50], disturbed sleep might be an underappreciated factor in the escalating rates of anxiety disorders.

Shifting to prevention, our findings suggest that even modest improvements in sleep quality may have the potential to reduce subjective anxiety, serving as a non-pharmacological prophylactic. That is, sleep may be seen as a modifiable risk factor and intervention target for those suffering from anxiety, both clinical and subclinical.

Methods

In-laboratory experiment. *Participants.* Eighteen healthy adults, aged 18–24 years (mean, 20.2 ± 1.5 years, nine women) completed a repeated-measures cross-over design (described below). Participants abstained from caffeine and alcohol for 72 h before each study session. Participants' habitual sleep–wake rhythm was monitored for the three nights before study participation, verified by sleep logs and actigraphy (a wristwatch movement sensor, sensitive to wake and sleep states; average sleep duration, 8.24 h ± 38 min). Data from these participants were also published in our recent work[70]. Exclusion criteria, assessed using a prescreening questionnaire, included: a history of sleep disorders, neurological disorders, closed head injury, Axis 1 psychiatric disorders, history of drug abuse and current use of antidepressant or hypnotic medication. Participants who reported sleeping <7 h per night or consuming three or more daily caffeine-containing drinks were also excluded from entering the study. The study was approved by the local human studies committee of the University of California Berkeley, with all participants providing written informed consent.

Experimental design. Following successful completion of screening, participants entered a repeated-measures study design (Fig. 1a), including two sessions conducted in a counterbalanced order—one after a normal night of sleep and one after 24 h of total sleep deprivation. Participants were randomly assigned to start with either a SD ($n = 8$) or a SR session ($n = 10$). Anxiety states were measured twice in each session using the state version of the STAI[71] (see Anxiety assessment–in-laboratory study)—in the evening before any sleep manipulation (between 20.00 and 22.00) and in the morning following both sleep sessions (08.00–09.00).

In the SD session, participants arrived at the laboratory at 21.30 and were continuously monitored throughout the enforced waking period by trained personnel. During the SD period, participants engaged in a limited set of activities such as Internet, email, short walks, reading, watching movies or playing board games. The following morning at approximately 10.30 (±45 min), participants performed the affective fMRI paradigm inside the scanner (details below). In the SR session, participants arrived at the laboratory at 19.00 and were wired up for an ambulatory PSG recording (detailed below) after which they were sent home, allowing for more naturalistic sleep. The next morning, participants returned to the laboratory and had the electrodes removed. Participants then performed the same activities as those described above in the SD condition, starting at the same circadian time. The SD and SR sessions were separated by at least 7 d, with their order counterbalanced across participants.

fMRI affective paradigm. During fMRI scanning, participants viewed 16 experimentally controlled video clips depicting aversive scenarios (featuring humans, animals and/or the environment in a counterbalanced manner) that induce robust affective brain activity in our a priori networks of interest[31,72]. Each video lasted 32.3 s on average (±2.5 s). Twelve additional videos of equivalent duration were created that depicted neutral scenarios related to common objects (for example, the history of typewriters or differences in envelope design). As with previous such fMRI paradigms, these neutral videos provided an on-task comparison to the emotional videos, specifically allowing for the discrimination of brain activity that is unique to the processing of emotional stimuli (emotional > neutral condition). The fMRI affective paradigm included eight emotional and six neutral videos in each experimental session. This choice was driven by the need for increased sampling of the emotional condition, accommodating for known higher variability in affective brain regions relative to neutral[73], thus improving signal sampling.

The paradigm had two versions, each including a different set of emotional and neutral videos. Participants were counterbalanced in the version of the paradigm they viewed such that each version was viewed in a SR session for half of the participants and in a SD session for others. Thus, video-clip themes were also counterbalanced across participants. In each session the videos were presented in two runs, with each run containing seven videos. The different video trials (emotional, neutral) appeared in randomized order within each run.

To verify attention to each video, participants were asked a brief memory question about the content, at the end of each clip (for example, 'How many children were featured in the video?' or 'What material was used to seal the first envelopes?'), followed by an inter-trial fixation period (jittered, 4–8 s). The start of each run contained a 10-s fixation block, allowing for steady-state equilibrium of the blood-oxygenation-level dependent (BOLD) fMRI signal.

Anxiety assessment—in-laboratory study. State anxiety was measured twice in each in-laboratory session using the validated 20-item state verson of STAI[71]. This inventory measures transient feelings of anxiety and ranges in score from 20 to 80, with higher scores indicating greater anxiety[71]. Response options used a four-point scale ranging from 'Not at all' to 'Very much so'. Sample items include 'I feel strained', 'I am worried' and 'I am jittery' as well as 'I feel steady' and 'I am relaxed', which are coded using an inverted scale (see Extended Data Fig. 2 for means and s.d. of all individual items). Cronbach's alpha for all 20 items was 0.88 on the SR session and 0.92 on the SD session.

In-laboratory PSG replication study. A second PSG study was conducted to validate, and to attempt to replicate, the original PSG findings using a similar cohort composition and, furthermore, was performed using in-laboratory sleep recordings to examine whether the original at-home recording environment was a factor. Here, 32 healthy adults, aged 18–24 years (mean, 20.47 ± 1.8 years, 18 women) took part in the overnight sleep study. Participants arrived in the laboratory at approximately 20.00 and were prepared for an overnight PSG sleep-recording session. Similar to the original cohort, participants were asked to report their current subjective anxiety using the state version of STAI[71] at two different times—in the evening before overnight sleep (20.00–22.00) and in the morning following sleep (08.00–09.00). Mirroring our original study, analysis focused a priori on the association between NREM SWS and SWA and the next-day reduction in anxiety.

Online micro-longitudinal studies 1 and 2. *Habitual variations in sleep associated with changes in anxiety.* In addition to the in-laboratory total sleep deprivation, we tested whether more modest night-to-night variability in sleep quality, focusing a priori on sleep efficiency, would predict day-to-day changes in subjective feelings of anxiety.

In online study 1, a total of 293 participants (mean age 36.84 years, 161 women) signed up for this study using Amazon Mechanical Turk (MTurk)—a platform where individuals can perform online tasks for a specified reimbursement (here, US$1.80). Enrolment was restricted to those with Internet Protocol addresses in the United States, and a previous online MTurk approval rating of 95% or higher. Following recruitment, participants were asked to complete sleep surveys quantifying their sleep across two consecutive nights (see Supplementary Table 2), followed by next-day assessment of anxiety using a short form of the STAI[74] questionnaire (see Anxiety assessment—online studies, below, for more details). In addition to the detailed information obtained from daily sleep logs, participants were also asked, 'how well did you sleep last night?' on a scale from 1 (extremely poor) to 5 (extremely good) as a subjective measure of sleep quality.

All questions were presented in random order. To measure anxiety in relation to previous sleep efficiency, the survey was available online only during a specific

time window in the morning (until 13.00), and participants were requested to complete the survey as close as possible to their wake-up time. Based on these quality control factors, and the repeated nature of the survey, not all recruited participants completed both daily measures or were eligible for the final analysis ($n = 75$). Additionally, 24 participants either had incomplete data, completed the survey at the wrong time or had duplicate entries of their survey data (for example, completed the same survey multiple times). The final sample therefore included 194 participants (mean age, 37.03 ± 11.3 years, 105 women).

In the second online study, a total of 187 participants (mean age, 36.56 ± 11.5 years, 85 women) signed up for the study using MTurk, with criteria similar to those detailed above. However, two key changes were made for this online study: (1) participants were asked to complete sleep surveys quantifying their sleep across four, instead of two, consecutive nights; and (2) next-day assessment of anxiety used both the short form of the STAI questionnaire (as in online study 1) and the BAI[44] (see Anxiety assessment—online sudies, below, for more details). Participants that failed to complete at least three daily surveys ($n = 33$) were excluded from further analysis to allow for sufficient variability in assessing directionality effects. The final sample included $n = 154$ participants (mean age, 36.78 ± 11.7 years, 68 women).

Analysis focused a priori on sleep efficiency, given previous studies linking anxiety with impairments in sleep quality and continuity relative to sleep duration alone[7,22,24]. Sleep efficiency was calculated using participants' daily sleep surveys, based on the percentage of time asleep out of total sleep duration (that is, total sleep time minus sleep latency and time spent awake after sleep onset[75]). Thereafter, we tested whether night-to-night variability in sleep efficiency, within participants, predicted day-to day changes in their subjective anxiety status.

In online study 1, examining our specific categorical hypothesis and providing a homologue to the in-laboratory study of a binary sleep condition separation, we categorically parsed the sleep data of the online participants based on whether they experienced a minimal threshold of an increase ($n = 47$) or decrease ($n = 33$) in sleep efficiency from one night to the next (above or below 3% change, respectively, negating statistical bias of zero-change influence on the outcome[75,76]). Thereafter, a comparison of the corresponding change in anxiety within individuals, from one day to the next, was performed. In online study 2, multilevel mixed models were used to examine the impact of night-to-night variability in sleep efficiency to subjective feelings of anxiety across multiple days. Briefly (but see Data analysis, below) two key models were tested: (1) the impact of a relative change in sleep efficiency within an individual to anxiety the next day ($\text{anxiety}_{(\text{day } n)} \sim \text{sleep efficiency}_{(\text{night } n)}$) and (2) the impact of a relative change in sleep efficiency to anxiety the next day while controlling for the impact of previous anxiety ($\text{anxiety}_{(\text{day } n)} \sim \text{sleep efficiency}_{(\text{night } n)} + \text{anxiety}_{(\text{day } n-1)}$). Of note, both online studies focused on assessment during weekdays to avoid known effects of weekend on sleep duration[77]. Moreover, we found no evidence for a change in either sleep efficiency ($F(1, 419.78) = 2.36$, $P = 0.12$) or anxiety ($F(1, 446.51) = 0.24$, $P = 0.6$) over the four different surveyed weekdays in online study 2, suggesting that day of week was not a significant modulating factor influencing our key variables of interest.

Anxiety assessment—online studies. Two anxiety assessments were used in each daily survey of the online micro-longitudinal studies. The first, a short form of the STAI questionnaire[74], was used in online studies 1 and 2. This questionnaire includes six statements similar to those described above for the in-laboratory STAI questionnaire (see Anxiety Assessment—in-laboratory study and Extended Data Fig. 3 for means and s.d. of all individual items). Cronbach's alpha for all six items was 0.82 on the first day of online study 1 and 0.86 on the first day of online study 2. The second anxiety assessment, BAI[44], was added to online study 2. The BAI is a 21-item self-report measure of anxiety that focuses on somatic content and includes symptoms such as 'Heart pounding/racing', 'Fear of worst happening' and 'Terrified or afraid'. Participants were asked to state how much they were bothered by each symptom since waking up that day, using a four-point scale ranging from 'Not at all' to 'Severely, it bothered me a lot' (see Extended Data Fig. 4 for means and s.d. of all individual items). Cronbach's alpha for all 21 items was 0.94 on the first day of online study 2.

Mood assessment (in-laboratory and online studies). Negative mood and anxiety are often co-morbid[41], and both affective states have been linked to lack of sleep[43]. We therefore controlled for co-occurring changes in mood in both the online and in-laboratory studies. In the latter, changes in mood were measured at the same circadian time as the anxiety assessment using the positive and negative affective scale (PANAS[78]). The PANAS is a 20-item self-report measure of current subjective mood that comprises two scales: Positive (for example, 'Excited', 'Strong') and Negative (for example, 'Ashamed', 'Distressed'). Participants are asked to rate the extent to which they were experiencing each item, using a five-point scale (1, 'very slightly or not at all'; 5,'extremely'). In online studies 1 and 2, changes in mood were tracked in each daily measure using the short form of the PANAS questionnaire[79]. This form of the PANAS includes a subsample of ten items (five negative, five positive) from the 20-item PANAS mentioned above, rated using the same five-point scale.

Data analysis. *Statistical analyses—in-laboratory study.* To test the hypothesis of greater levels of anxiety following sleep loss, a repeated-measure ANOVA was calculated using SPSS (IBM Corp.), taking into account time (evening and morning anxiety scores) across the different sleep conditions (SR, SD). In case of significance, post hoc tests were computed using two-sided *t*-tests corrected for multiple comparisons using Bonferroni correction. For each post hoc comparison, we also report the partial eta-squared as a measure of effect size. Sample size of the in-laboratory study was not predetermined statistically as it followed sample sizes matched to effects sizes reported in previous experimental sleep studies examining anxiety[46,55,80].

To assess the hypothesis that this anxiogenic impact of sleep loss involved altered activity in four a priori ROIs (mPFC, insula, amygdala and dACC), condition-specific ROI activity was extracted using recommended neuro-imaging standards[81], comparing mean activity across the entire ROI sphere between sleep and task conditions. For each region, a repeated-measure ANOVA with the factors sleep (SR/SD) and task condition (emotional/neutral clips) was applied. Only in cases of a significant interaction between task condition and sleep was ROI activity considered for further analysis. For all ANOVA testing, sphericity was examined using Mauchly's test and, in cases of violation, *F*-values were adjusted using the Greenhouse–Geisser correction.

In the case of null results, we conducted a Bayes factor analysis to determine the relative strength of the null hypothesis using JASP (JASP Team, 2017). The Bayes factor overcomes some of the issues associated with null hypothesis significance testing, by quantifying the relative likelihood of the data under the null versus the alternative hypothesis. Specifically, for Bayesian alternatives to the *t*-test or regression we calculated the Jeffrey–Zellner–Siow[82] Bayes factor with an effect size of 1 to determine the strength of evidence against a group-level difference in brain activity or behaviour. All Bayesian analysis of variance used the default settings in JASP. A Jeffrey–Zellner–Siow Bayes factor can be interpreted such that a value of 3 favours the null hypothesis three times more than the alternative hypothesis, while a value of one-third favours the alternative three times more than the null hypothesis.

Associations between ROI activity and behavioural measures of anxiety or sleep were tested using Pearson's correlation, with mean activity from the entire literature-defined spheres (that is, limited not only to the activated cluster) to avoid spurious fMRI–behaviour correlations[81]. All brain and behaviour measures (including sleep-related measures) were tested for normality using the Shapiro–Wilk test before parametric statistic testing.

Statistical analyses—online micro-longitudinal study. The hypothesis of the micro-longitudinal study stated that changes in sleep quality within an individual, from one night to the next, predicted day-to-day changes in anxiety. In online study 1, two independent analysis approaches were taken to test this hypothesis: (1) mixed ANOVA applied to morning anxiety scores as a factor of sleep efficiency (worse/improved sleep efficiency subgroups, see above) and time (day 1/day 2), with main and interaction effects tested at a significance of $P < 0.05$; and (2) across all study participants, the association between sleep efficiency and anxiety was tested using Pearson's correlation, similarly assessed at a significance of $P < 0.05$. Sample sizes of both online studies were not predetermined statistically, as they followed sample sizes matched to effects sizes reported in previous habitual sleep studies examining anxiety[40,83].

In online study 2, data consisted of up to four data points nested within each individual. Because these nested data violate assumptions of independence, we analysed our data using multilevel mixed modelling with participants as random effects and day as a repeated measure (Mixed Models, SPSS v.25). In all key models, predictors were person-centred so that results would reflect changes for each participant from their own average (that is, having worse or better sleep relative to an individual's average sleep). Additionally, to explore the directional impact of changes in sleep efficiency on anxiety, lagged day analyses were conducted by regressing anxiety on day *n* onto both sleep efficiency the previous night and anxiety on day *n*–1. For all models, degrees of freedom were calculated using the Satterthwaite approximation, which yields degrees of freedom that are somewhere between the number of repeated measures and the number of individuals. Total variance explained (R^2) for online study 2 was calculated using the recommended proportional reduction in variance approach[84,85]. In this method $R^2 = 1 - \sigma^2/\sigma_0^2$, where σ^2 is the full model residual variance and σ_0^2 is the null model residual variance.

fMRI acquisition and analysis. BOLD contrast functional images were acquired with echo-planar T2*-weighted imaging using a Siemens 3 Tesla MRI scanner with a 12-channel head coil. Each image volume consisted of 37 descending 3.5-mm slices (matrix = 96 × 96, TR = 2,000 ms, TE = 22 ms, voxel size = 3.5 × 3.5 × 3.2 mm^3, flip angle = 50°, interslice gap = 0.3 mm). One high-resolution, T1-weighted structural scan was acquired at the end of each session (matrix = 256 × 256, TR = 1,900, TE = 2.52, flip angle = 9°, field-of-view = 256 mm, voxel size = 1 × 1 × 1 mm^3).

Preprocessing and data analysis were performed using Statistical Parametric Mapping software implemented in Matlab (SPM12; Wellcome Department of Cognitive Neurology, London, UK). Images were motion corrected and slice time corrected, and then spatially normalized to the Montreal Neurological Institute template and smoothed using a 6-mm, full-width-at-half-maximum Gaussian

kernel using default parameters in SPM12. For each subject, trial-related activity was assessed by convolving a vector of trial onsets with a canonical haemodynamic response function.

The 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 for modelling movement-related artefact in the time series. To further address the influence of motion on BOLD data, we calculated frame-wise displacement of head motion based on the motion parameters estimated during preprocessing using the ArtRepair toolbox[86]. TRs including frame-wise displacement values >1 were interpolated with the nearest artefact-free TRs surrounding the motion. To control for physiological noise, five principal components of cerebrospinal fluid (CSF) and white matter signal were added as regressors to the design matrix, implemented through the CompCor pipeline[87]. 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.

Following preprocessing, a general linear model (GLM)[88] was specified for each participant to investigate the effects of interest. Contrasts were created at the first level focusing on emotional versus neutral contrast to target regions sensitive to affective processing. The resulting contrasts were then taken through to a second-level, random-effects analysis, to assess group-level effects, examined using a paired *t*-test (SR < > SD). Analyses focused a priori on activity in a set of brain regions comprising the extended limbic network, which have been implicated in studies of both anxiety disorders[10,69] and affective processing in highly anxious individuals[8,12,38]. These regions include: bilateral amygdala[13–15], bilateral insula[10,16,89], dACC[9,15,89] and medial prefrontal regions[18,90–92]. ROIs were an independent set of literature-defined regions, constructed as 5-mm spheres around reported coordinates for each region, selected to approximate the average cluster sizes reported in the original studies from which coordinates were taken (see Supplementary Table 1 for a complete list of ROIs). Beyond these a priori ROIs, non-a priori whole-brain results are provided in Supplementary Table 3 for completeness, but are not discussed further.

To identify changes in functional connectivity of the mPFC–amygdala circuit as a function of sleep, a psycho-physiological interaction (PPI) analysis was conducted separately for each session (SD/SR) using SPM12 (ref. [93]). PPI analysis examined connectivity between the mPFC ROI seed and the amygdala, using the same a priori ROIs chosen for the GLM analysis above. Consistent with standard PPI practices[93], 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.

These matrices were defined separately for each sleep condition, and further included all nuisance regressors used in the GLM analysis (for example, movement and physiological noise as detailed above[87]). A second-level paired *t*-test comparing the two conditions (SD, SR) was then applied to PPI results.

Sleep recordings and spectral analysis. Sleep was recorded using standard PSG including EEG, electromyography and electrooculography recordings. EEG was recorded from 13 scalp electrodes (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4 and Oz; International 10–20 System), referenced to left and right mastoid (A1, A2). For the in-laboratory replication PSG study, scalp electrodes also included temporal electrodes (T3, T4, T5 and T6) as well as O1, O2 and Pz. EEG signals were sampled at 200 Hz in the original study and 400 Hz in the in-laboratory PSG study. PSG recordings were scored according to standard criteria[94]. Sleep statistics are provided in Extended Data Fig. 5, and conform to population norms for this age range[75].

All EEG analyses were performed in MATLAB 8.6 (The MathWorks), including the add-in toolbox EEGLAB (http://sccn.ucsd.edu/eeglab/). Power spectral analysis of sleep EEG was performed according to previously published methods[27]. Specifically, raw EEG channels were filtered with high- and low-pass finite impulse response at 0.5 and 50 Hz, respectively. Artefacts were then visually rejected in 5-s epochs and removed from subsequent analyses. One subject was excluded from spectral analysis due to a limited number of artefact-free NREM epochs (<40%). Power spectral density was calculated with a fast Fourier transform (FFT) on each hamming-windowed 5-s epoch. FFT results were then sorted according to sleep stage and averaged for each respective stage.

Band power was calculated by averaging across standard EEG frequency band ranges: δ (0.8–4.6 Hz), θ (4.8–8.0 Hz), α (8.2–12 Hz), σ (12.2–15 Hz), β_1 (15.2–20 Hz), β_2 (20.2–35 Hz) and γ (35.2–45 Hz). Values were further divided by total power across all bands to yield relative spectral measures per sleep stage. We focused a priori on NREM sleep due to evidence linking changes in this sleep stage to both clinical and non-clinical states of anxiety[22–24,26,27], and on NREM SWA power (δ, 0.5–4 Hz), based on previous clinical studies reporting alterations in slow-wave frequencies in anxious patient cohorts[29] and in highly anxious healthy individuals[28]. To examine NREM SWA power in relation to anxiety, relative power values were averaged across three channel derivations: Frontal (F3, F4, Fz), Central (C3, C4, Cz) and Posterior (P3, P4, Oz).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

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

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Author contributions

E.B.S and M.P.W. conceived and designed the study. E.B.S and A.R. collected the data. E.B.S, A.R. and M.P.W. analysed the data. E.B.S, A.G.H and M.P.W wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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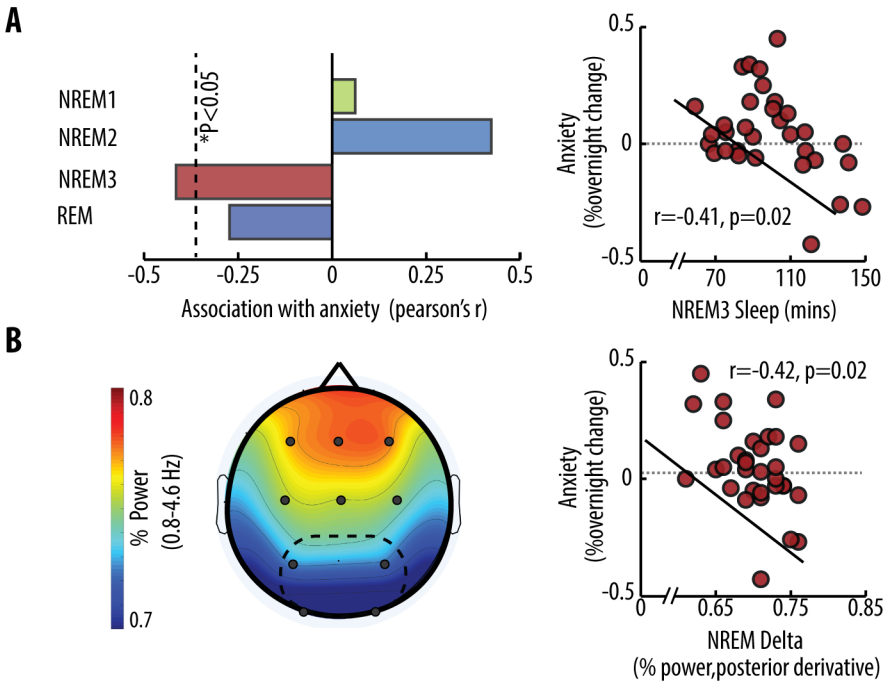

Extended Data Fig. 1 | Sleep rested physiology in relation to next-day anxiety (PSG replication study). (**a**), Anxiety association in relation to REM and non-REM sleep stages (left panel). Time spent in deep NREM sleep (NREM3) was associated with a significant reduction in next-day anxiety (right scatter plot). (**b**), Power in the Delta band (SWA, 0.8-4.6 Hz) during NREM sleep (left panel) was associated with lower morning anxiety (right scatter plot), most pronounced for posterior derivations (circled by a dashed line). Dashed grey lines denote zero crossing.

	Sleep Rested	Sleep Deprived		Sleep Rested	Sleep Deprived
Item 1	1.67 ± .84	2.06 ± .93	**Item 11**	2.06 ± .80	2.67 ± .90
Item 2	1.72 ± .82	2.00 ± 1.08	**Item 12**	1.44 ± .70	1.44 ± .51
Item 3	1.44 ± .85	1.56 ± .70	**Item 13**	1.22 ± .54	1.72 ± .75
Item 4	1.28 ± .46	2.22 ± 1.06	**Item 14**	1.17 ± .51	1.44 ± .61
Item 5	2.22 ± .80	2.83 ± .92	**Item 15**	2 ± .76	2.72 ± .89
Item 6	1 ± .00	1.22 ± .54	**Item 16**	2.06 ± .87	2.83 ± .85
Item 7	1.44 ± .61	1.11 ± .32	**Item 17**	1.28 ± .46	1.28 ± .57
Item 8	1.22 ± .42	1.11 ±. 32	**Item 18**	1.06 ± .23	1.28 ± .46
Item 9	2.33 ± .76	2.94 ± .80	**Item 19**	2.11 ± .96	2.83 ± .78
Item 10	2.11 ± .83	2.94 ± .80	**Item 20**	2.28 ± .95	2.83 ± .98

Extended Data Fig. 2 | STAI item values (In-lab Study). Item values for in-lab STAI-state questionnaire (mean ± SD, higher values indicate greater anxiety).

	Online Study 1	Online Study 2
Item 1	2.32 ± 0.9	2.39±0.98
Item 2	1.54 ± 0.72	1.4±0.72
Item 3	2.24 ± 0.95	2.22±0.92
Item 4	1.36 ± 0.71	1.35±0.68
Item 5	1.44 ± 0.76	1.39±0.74
Item 6	2.03 ± 0.92	2.06±0.93

Extended Data Fig. 3 | STAI item values (Online Studies). Item values for the online short STAI-state questionnaire (day 1; mean ± SD, higher values indicate greater anxiety).

	Mean ± SD		Mean ± SD		Mean ± SD
Item 1	0.28 ± 0.58	Item 8	0.31 ± 0.6	Item 15	0.19 ± 0.56
Item 2	0.44 ± 0.69	Item 9	0.18 ± 0.5	Item 16	0.16 ± 0.52
Item 3	0.24 ± 0.56	Item 10	0.33 ± 0.64	Item 17	0.15 ± 0.48
Item 4	0.33 ± 0.68	Item 11	0.15 ± 0.5	Item 18	0.25 ± 0.57
Item 5	0.25 ± 0.59	Item 12	0.16 ± 0.48	Item 19	0.26 ± 0.56
Item 6	0.24 ± 0.54	Item 13	0.23 ± 0.59	Item 20	0.22 ± 0.61
Item 7	0.20 ± 0.5	Item 14	0.13 ± 0.41	Item 21	0.26 ± 0.62

Extended Data Fig. 4 | BAI item values (Online Study 2). Item values for Beck Anxiety Inventory (day1; mean ± SD, higher values indicate greater anxiety).

	Time (min)	Percentage of total sleep time
Sleep latency	15.53 ± 13.07	
Total sleep time	398 ± 75.68	
WASO	26.82 ± 24.6	
Sleep Efficiency	88.96± 6.23	
NREM stage 1	29.91 ± 11.92	7.72 ± 3.10
NREM stage 2	202.79 ± 45.96	51.08 ± 7.58
NREM SWS	90.56 ± 33.04	23.28 ± 7.29
REM	74.76 ± 30.32	18.34 ± 5.67

Extended Data Fig. 5 | Sleep Characteristics (In-lab Study). Polysomnography sleep characteristics for the sleep-rested night (Mean ± SD). WASO, wake after sleep onset; NREM, non rapid-eye-movement sleep; SWS, slow-wave sleep (SWS, NREM stages 3 and 4); REM, rapid-eye-movement sleep.

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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).

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Life sciences study design

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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.

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☒	☐ 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