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Supplementary information

Overanxious and underslept

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Supplementary Information: Overanxious and Under Slept

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Supplementary Table 1: Regions of Interest for fMRI analysis

Extended Limbic Network ROIs				
X	Y	Z	Laterality	Label
−27 23	2 -3	-22 -21	Left Right	Amygdala
8	51	5	Mid	Medial PFC
7	−2	38	Mid	Dorsal anterior cingulate
-40 43	−3 0	3 8	Left Right	Insula

Supplementary Table 2: Daily Sleep Log

Sleep diary questions completed by participants of the micro-longitudinal online studies across two to four consecutive nights of habitual sleep

Q1.	What time did you go to bed last night?
Q2.	How long did it take you to fall asleep last night?
Q3.	What time did you wake up this morning?
Q4.	How many times did you wake up during the night?
Q5.	If you woke up during the night, how long did it take you to get back to sleep?

Supplementary Table 3: Exploratory whole brain analysis

Exploratory whole-brain analysis showing all peak activations (MNI coordinates, P<0.005, min 5 voxels for paired comparison; sleep rested <> sleep deprived) of the emotional vs. neutral clip parametric contrast. These non a-priori whole-brain data are provided simply for descriptive purposes, without inference. Cluster size is in voxels; voxel size is $3mm^3$.

Sleep Deprived > Sleep Rested

X	Y	Z	T	Anatomical Label	Cluster Size
9	5	38	5.41	R Mid Cingulate Cortex	31
0	-1	50	3.79	L Mid Cingulate Cortex	18
-30	5	-28	4.91	L Amygdala\ Medial Temporal Pole	5
-42	11	-22	4.11	L Medial Temporal Pole	5
57	-13	11	3.48	R Sup. Temporal \ Mid Insula	7
-45	-1	-25	3.48	L Middle Temporal Gyrus	5
57	-13	11	3.37	SMA	11
-36	-16	-16	3.66	L Hippocampus	5

Sleep Rested > Sleep Deprived

X	Y	Z	T	Anatomical Label	Cluster Size
48	-37	8	-4.91	R Superior Temporal Gyrus	31
54	-49	20	-4.77	R Superior Temporal Gyrus	50
45	-25	-16	-4.74	R Inferior Temporal Gyrus	10
-51	-49	26	-4.53	L Superior Temporal Gyrus	87
-6	50	-7	-4.06	L Medial Frontal Gyrus \Anterior Cingulate Cortex	40
-27	-82	-28	-4.02	L Cerebellum	8
-6	53	17	-3.85	L Superior Medial Frontal	7
51	5	-31	-3.77	R Inferior Temporal Gyrus	5

54	29	-1	-3.76	R Inf. Frontal Gyrus	5
-57	-49	8	-3.76	L Middle Temporal Gyrus	18
3	62	20	-3.60	Medial Frontal Gyrus	11
-9	-46	35	-3.50	L Posterior Cingulate Cortex	16
15	56	5	-3.32	R Superior Medial Gyrus	6
0	-94	17	-3.23	L Calcarine Gyrus	7

Supplementary Note 1: Mood fluctuations in relation to anxiety and sleep

(a) Mood, anxiety and brain activity: In-lab Study

Similar to the increase in anxiety, sleep deprivation triggered a change in positive and negative mood states. Relative to a sleep rested night, sleep deprivation decreased positive mood ($t(17)=-5.36$, $P<0.0001$) and increased negative mood ($t(17)=2.3$, $P=0.03$), consistent with prior reports[1,2]. We therefore examined whether these changes in mood obviated the association between anxiety and the change in brain activity (medial PFC). Specifically, we conducted a multiple regression model that included both mood and anxiety states as predictors for mPFC activity. This analysis revealed that sleep-loss associated changes in anxiety remained a significant predictor of mPFC activity when controlling for corresponding mood states ($\beta=-0.726$, $t(14)=-2.33$, $P=0.03$).

Lastly, we examined whether changes in mood across the sleep rested night similarly obviated the association between anxiety and NREM SWA. Here, too, NREM-SWA remained a significant predictor of overnight changes in anxiety from evening to morning when controlling for a corresponding change in mood (Frontal derivation: $\beta=-0.588$, $t(14)=-2.43$; Central derivation: $\beta=-0.674$, $t(14)=-2.95$; Posterior derivation: $\beta=-0.77$, $t(14)=-3.63$; all $P<0.05$). Together, these findings indicate that the reported anxiogenic impact of sleep loss and related changes in mPFC activity, as well as anxiolytic relationship with NREM sleep physiology, remain significant after changes in mood are accounted for.

(b) Mood, anxiety and sleep -Online Study 1 and 2

Anxiety is often comorbid with alterations in mood[3], and both have been associated with poor sleep[4,5]. We therefore examined the impact of mood states on the association of habitual sleep and anxiety reported in our Online Studies. Specifically, a multiple regression analysis was used, taking into account both mood and sleep efficiency as predictors of mean anxiety levels in both

Online Studies. This analysis revealed that when controlling for covariations in mood, sleep efficiency still significantly predicted anxiety levels across participants (Online Study 1: β=-0.146, t(188)=-2.93 ,P=0.004; Online Study 2: β=-0.214, t(139)=-3.11, P=0.002).

In addition to these control analyses, we added a secondary anxiety assessment; the Beck Anxiety Inventory to Online Study 2 (BAI[6]). The BAI was added to the second Online Study as it takes into account somatic measures of anxiety (e.g. Heart pounding/ racing", "Fear of worst happening" and "Terrified or afraid"), and is less collinear with mood states[7]. Analyses of the BAI data revealed that worse sleep efficiency was associated with greater subjective anxiety the following day, similar to the STAI findings (β=-13.49, 95% CI= [-21.32, -5.65], t(61.27)=-3.44, P=0.001). Notably, this effect was significant when controlling for corresponding fluctuations in mood (β=-7.91, 95% CI= [-13.95, -1.87], t(360.66)=-2.57, P=0.01). These findings demonstrate that the relationship between anxiety and habitual sleep remains significant when accounting for co-occurring fluctuations in mood.

Supplementary Note 2: In-lab PSG replication study

Results from our second in-lab PSG study (N=32) replicated our original findings. In this independent dataset, NREM SWS predicted the degree of overnight reduction in anxiety from evening to morning (R=-0.41, 95% CI=[-0.08,-0.67], P=0.02, **Extended Data Fig. 1a**), such that individuals with greater SWS expressed a greater evening-to-morning dissipation of anxiety. In addition, NREM SWA demonstrated a similar anxiolytic benefit as found in the main cohort: greater amounts of NREM SWA, especially in the same posterior topographical regions, predicted greater overnight reduction in anxiety (**Extended Data Fig. 1b,** Posterior derivations: R=-0.42, 95% CI=[-0.81, -0.61], P=0.02).

In addition to replicating the original hypothesis, the second PSG study also served to address the impact of sleep location on our findings. In the original cohort, participants in the sleep rested session were sent home to allow for more naturalistic sleep conditions, while the sleep deprived session was conducted in lab to allow for overnight participant supervision (see Methods). It is therefore possible that a night of sleep at home served as a less anxiogenic state than a night spent in the lab above and beyond the impact of sleep loss. Addressing this issue, it is first relevant to note that all measures of anxiety, regardless of sleep condition, were conducted in the laboratory. Therefore, participants' location (which was consistent across conditions) may be less likely to confound measures of anxiety in the original cohort. Still, to corroborate this assumption, we asked participants in the second PSG study to stay in the laboratory for a night of PSG recorded sleep, while we measure their anxiety before and after sleep.

Results from this study mirror our original findings, and demonstrate very similar morning anxiety levels to the values collected in our original study (Original Study (home): 33.22 ± 7.8, Second PSG Study (in-lab): 31.78 ± 9.6; $t(48)=-0.54$, $P=0.6$). This was similarly true when comparing overall change in anxiety from evening to morning (Original Study (home): -0.27 ± 4.4; Second PSG Study (in-lab): 1 ± 5.8; $t(48)=-0.8$, $P=0.4$). Together, these data indicate that sleep location (home vs. in-lab) does not appear to significantly impact the reported anxiety effects, and suggest the anxiogenic impact of sleep loss is robust to study context (home vs. in-lab sleep recording).

Supplementary Note 3: daily changes in anxiety in relation to sleep duration and subjective sleep quality

The main study hypothesis focused a priori on sleep efficiency as associated with anxiety, given previous studies linking anxiety with measures of overall sleep quality, relative to sleep duration alone[8-10]. In addition to our a-priori variable of sleep efficiency we further examined whether sleep duration is associated with next-day anxiety across both online studies. In Online Study 1, we found no significant association between anxiety fluctuations from one day to the next and the corresponding change in sleep duration ($R=-0.063$, $P=0.4$, $N=194$). Similar results were found in Online Study 2, where a relative change in sleep duration from one night to the next was not predictive of next-day anxiety ($\beta=-0.001$, $t(74.62)=-0.58$, $P=0.56$).

Next, we examined the association of next-day anxiety with subjective sleep quality. As noted in the manuscript, subjective sleep quality was assessed in each daily measure by asking participants to rate the overall quality of their sleep last night using a scale ranging from 1 ("Extremely Poor") to 5 ("Extremely Good"). Of note, subjective sleep quality was highly correlated with our key measure of sleep efficiency across both online studies (Study 1: Rho=0.58, N=191; Study 2: Rho=0.57, N=154, both $P<0.0001$, values averaged across days for each participant). This finding indicates that both measures accurately capture sleep quality. As expected from the relationship with sleep efficiency, changes in sleep quality from one night to the next similarly predict changes in next-day anxiety across both Online Studies (Study 1: R=-0.26, N=194, P=0.0002; Study 2: β=-1.12, $t(93.68)=-7.51$, $P<0.0001$). These findings lend further support to the original hypothesis: nightly impairments in sleep quality as linked to greater anxiety the next day.

Supplementary Note 4: the directional influence of the anxiety-sleep 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. To estimate the unique contribution of sleep to next-day anxiety, we further examined the impact of anxiety on subsequent sleep efficiency while controlling for prior sleep efficiency. Though our study was not designed to address the impact of anxiety on subsequent sleep, this inverse model can help describe the bidirectional association between poor sleep and anxiety. We therefore used the longer sampling period of Online Study 2 to examine how sleep efficiency on night N+1 is predicted by subjective anxiety the previous day (day N), when controlling for prior sleep efficiency (night N). That is, is there an impact on the quality of subsequent sleep on days of greater subjective anxiety, when prior sleep status is also taken into account. The results of this analysis revealed that anxiety was not a significant modulator of subsequent sleep efficiency (β=0.003, $t(236.18)=0.35$, P=0.72) when controlling for prior sleep efficiency (β=0.73, $t(252.25)=5.65$, $P<0.0001$). These data do not negate a bidirectional association between poor sleep and anxiety. Instead, they simply show that prior sleep efficiency robustly impacts subjective levels of next-day anxiety, beyond preexisting anxiety states.

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