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Association of naturally occurring sleep loss with reduced amygdala resting-state functional connectivity following psychosocial stress

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ABSTRACT

Enduring sleep loss is a risk factor for a variety of both somatic and mental health issues. When subjected to sleep loss, the brain becomes vulnerable to critical alterations in cognitive and emotional processing. In our study, we examined the effect of psychosocial stress on amygdala resting-state functional connectivity in participants with cumulative sleep loss calculated across the seven days preceding scanning. For this purpose, forty-five healthy male participants completed a one-week sleep diary and underwent resting-state scans before and after taking part in the ScanSTRESS paradigm, which allows social stress induction during functional magnetic resonance imaging. Sleep loss was negatively associated with seed-based functional connectivity of the left amygdala with the medial prefrontal cortex, dorsal anterior cingulate cortex, anterior insula, posterior cingulate cortex, and dorsolateral prefrontal cortex. That is, participants with higher amounts of sleep loss showed reduced left amygdala connectivity after social stress induction to cortical regions encompassing main nodes of the brain's default mode network and salience network. Our results shed more light on how brain functional connectivity may shape the brain's stress response in the context of naturally occurring sleep loss, revealing a potential neural mechanism for increased vulnerability to stress-related psychopathology.

1. Introduction

When we sleep less than biologically determined, our brain becomes vulnerable to cognitive malfunctioning: According to a number of neuroimaging studies, acute sleep deprivation causes critical changes in brain activity and functional connectivity in regions associated to a variety of cognitive domains, such as attention, working memory, incentive processing, and memory consolidation (Krause et al., 2017). At the same time, sleep disturbances are found to be prevalent in almost any kind of mental disorder (Baglioni et al., 2016). Not only are sleep disorders, such as insomnia, thought to affect dopaminergic signaling in substance use disorders (Brower et al., 2001; Hasler et al., 2016) and emotional processing in post-traumatic stress disorder (PTSD) (Germain, 2013), they also constitute a severe risk factor for the development of major depression (Baglioni et al., 2011). Taking into

account additional somatic health consequences, including increased risk for coronary heart disease, obesity, diabetes, cancer, but also for occupational accidents (Kecklund and Axelsson, 2016), sleep loss can be regarded as a potent stress factor.

A key brain region in the stress response is the amygdala, which is believed to play a leading role in threat and, more general, salience detection (LeDoux, 2003). Imaging studies suggest that the amygdala is predominantly activated by arousing emotional stimuli, both of negative (e.g., fear) and positive valence (e.g., humor), including social emotions (e.g., embarrassment) (Costafreda et al., 2008). On the other hand, connectivity of the amygdala with other stress-related brain areas is typically strengthened after stress induction. For instance, enhanced amygdala resting-state functional connectivity (RSFC) to areas of the salience network (SN) has been found in the immediate aftermath of social stress (van Marle et al., 2010), while increased RSFC with core

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regions of the default mode network (DMN) was found up to an hour after termination of stress, hypothesized to reflect emotional memory formation and regulatory feedback during stress recovery (Veer et al., 2011). More recently, delayed recovery of amygdala-hippocampal RSFC was associated with lower levels of cortisol (Vaisvaser et al., 2013), whereas amygdala-precuneus RSFC was found to predict the extent of behavioral stress recovery, as indicated by a decrease in subjective stress experience (Maron-Katz et al., 2016).

In a study where participants were subjected to 35 h of total sleep deprivation, amygdala hyperactivation and reduced amygdala functional connectivity to the medial prefrontal cortex (MPFC) was found in response to aversive stimuli (Yoo et al., 2007). This pattern has since been replicated for both negatively and positively valenced visual stimuli (Goldstein et al., 2013; Gujar et al., 2011; Motomura et al., 2013), which suggests that acute forms of sleep loss induce a biased reactivity of the amygdala towards emotionally arousing events. Moreover, increased amygdala activity and reduced amygdala-prefrontal functional connectivity following acute sleep deprivation have been associated with increased emotional distractibility in the context of working-memory performance (Chuah et al., 2010), which closely follows effects of acute social stress (Oei et al., 2012). Studies investigating more commonplace forms of sleep loss yielded comparable results. For instance, poor sleep quality over the course of one month was found to moderate the association between amygdala reactivity to fearful faces and the extent of stress and depression symptoms (Prather et al., 2013). Also, self-reported sleep duration of the preceding night was shown to be negatively correlated with amygdala RSFC to the ventromedial prefrontal cortex (VMPFC) (Killgore, 2013), suggesting that stronger negative RSFC may represent a more stable prefrontal top-down regulatory functioning when having slept for a longer time.

So far, chronic sleep loss has not been linked to amygdala functional connectivity *after* stress exposure, in spite of the impact this might have on our everyday emotional well-being. To this end, we recruited male participants with a tendency to accumulate sleep loss in their daily lives to explore how sleep loss may be associated with connectivity in stress-related brain circuits after exposure to psychosocial stress. We hypothesized that the strength of amygdala RSFC after stressor exposure would be associated with the amount of sleep loss accumulated in the week prior to scanning.

2. Methods

2.1. Participants

Fifty males aged between 20 and 48 years ($M = 30.23$, $SD = 5.39$) were recruited, meeting the following inclusion criteria: (1) a regular Monday-to-Friday work week of 30–50 hours with weekend days off, (2) no shift-work, (3) a late chronotype conflicting with early start of work, (4) no current or past psychiatric disorders, (5) non-smoking. Of the 50 participants taking part in the scanning sessions, five were excluded because of an incidental finding in their structural scan ($n = 3$) or missing sleep diary data ($n = 2$). The final sample comprised 45 participants (age 20–48 years, $M = 29.271$, $SD = 4.84$, three left-handed) whose data were submitted to further analysis. Scanning took place either on Wednesday ($n = 25$) or Thursday evenings ($n = 21$) between 5 pm and 10 pm. Participants refrained from caffeine two hours before scanning and from physical activity for the whole day. The study was approved by the ethics committee of Charité – Universitätsmedizin Berlin. All participants provided written informed consent and were paid for their participation.

2.2. Sleeping parameters

For screening purposes, participants took part in a 45-minute telephone interview, which included the German version of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (Wittchen et al.,

1997), as well as the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al., 2004). In order to estimate the participants' chronotype (i.e. early, normal, late type) the following sleep parameters were assessed from the MCTQ (see also Supplemental Table S-1 for a list of the computed variables): (1) number of work days/free days per week (WD, FD), (2) sleep onset on work days/free days (SO_w , SO_f), (3) sleep end on work days/free days (SE_w , SE_f), (4) average weekly sleep duration (SD_{week}), and (5) mid-point of sleep on free days (MSF). As people tend to compensate cumulative sleep loss from work days with extra sleep on free days, MSF was adjusted (mid-point of sleep on free days, sleep corrected; MSF_{sc}) in order to represent a less confounded marker of chronotype (Roenneberg et al., 2004). If the average sleep duration on free days (SD_f) was shorter than or equal to the average sleep duration on work days (SD_w), MSF was used as a proxy of chronotype. However, if SD_f was greater than SD_w , the following formula was used: $MSF_{sc} = MSF - (SD_f - SD_{week})/2$. As cut-off for late chronotypes, an MSF_{sc} of 4:30 a.m. or later was set.

Finally, habitual sleep loss from the MCTQ (Sloss [MCTQ]) was estimated: If SD_{week} was greater than SD_w , the formula was: $(SD_{week} - SD_w) \times \text{number of work days}$. If SD_{week} was equal to or shorter than SD_w , the formula was: $(SD_{week} - SD_f) \times \text{number of free days}$. Additionally, all recruited participants were asked to complete a seven-night sleep diary during the week before the scanning session. From the sleep diary data, the amount of sleep loss during the week preceding scanning (Sloss [diary]) could be estimated, using the same formula as described above. Both measures of Sloss were assessed to test whether effects would be more pronounced for trait-like (MCTQ) or state-like (diary) sleep loss. A table of the sleeping parameters collected in the sleep diaries can be found in the Supplement (Table S-2).

2.3. Stress induction

An adapted version of the ScanSTRESS paradigm (Streit et al., 2014) was used to induce stress. The paradigm includes two interchanging types of blocks: a stressful 'performance phase' with social evaluation and a control 'relaxation phase'. The 'performance phase' consisted of two different tasks inside the scanner: A mental rotation task followed by an arithmetic subtraction task (see Fig. 1). During mental rotation blocks, a three-dimensional geometrical figure was presented in the upper part of the screen. Below this figure, the matching, but rotated figure had to be chosen correctly from three alternatives. For the arithmetic subtraction blocks, the number 13 had to be subtracted from a four-digit number presented on screen. The correct result had to be chosen from four alternatives below. A time limit was set for each item, which was continuously adapted to the participant's current performance speed. This ensured that participants with higher performance rates were faced with similar task demands as participants with lower speed. In addition, intermediate comments appeared on the screen in case the participant's performance became too slow ("Work faster!") or a wrong answer was given ("Incorrect!"). Furthermore, participants were observed by an expert committee, which consisted of a female and a male member of the study team seated in the control room, dressed in a lab coat, and keeping their facial expressions neutral all the time. A live video of the committee was visible on the screen inside the scanner alongside the task. It is this social-evaluative threat component that was shown to be most effective in eliciting cortisol elevations in response to a stressor (Dickerson and Kemeny, 2004). The control 'relaxation' blocks followed the two 'performance' blocks and consisted of a similar, but cognitively less demanding task. Figures had to be matched to their not-rotated counterpart, while the four-digit number presented above simply had to be matched with one of four numbers below. In addition, the live video of the expert committee was made opaque and crossed out, while the observers avoided looking into the camera. The adapted ScanSTRESS paradigm was programmed and presented with Presentation software (Version 18.1, www.neurobs.com).

Before going into the scanner, participants received a detailed

Fig. 1. The scanning procedure (A) comprised a T1 MPRAGE at the start, two resting-state (RS) scans (one before and one after the stress task), followed by a further task scan (i.e., a delayed match-to-sample task with emotional distraction). Saliva samples (S) were taken at four time points (min), relative to the beginning of the stress induction: t1 = pre-scan, t2 = post resting-state 1, t3 = post-stress, t4 = post resting-state 2. Three more saliva samples were taken: After a second task, and before and after the debriefing. HR(V) = heart rate and heart rate variability. The ScanSTRESS paradigm (B) consisted of two runs inside the MRI scanner: a practice run without scanning (duration: 2:50 min) and an experimental run during scanning (duration: 11:00 min).

instruction in a separate preparation room and were asked for strong performance and concentration on the task in order to achieve the best possible results. Before entering the scanning room, participants were briefly introduced to the expert committee and noted a video in one corner of the control room that showed the head of a person lying in the scanner, creating the impression that participants could be observed from outside the scanner room. After the first resting-state scan, a practice run of the task took place without scanning, which consisted of a control block and a performance block with epochs of 30 s followed by breaks of 10 s. After that, a verbal feedback was given by the female member of the expert committee, noting that the participant's current performance was subpar and had to improve for the following run to not endanger the outcome of the experiment. Importantly, opposite-sex stress panels (with respect to the participant) have shown to give stronger stress responses (Duchesne et al., 2012). After the practice run and feedback, the scanner was started and both performance and control phases were administered during the experimental run. This time, each block lasted 60 s and was followed by a break of 20 s. After the scanning session, participants received a debriefing in which the task procedure and its aim were revealed.

2.4. Assessment and statistical analysis of cortisol, stress ratings, and heart rate (variability)

In order to detect participants' levels of cortisol due to the social stress induction, seven saliva samples were taken at different time points in the course of the experiment (see Fig. 1 for detailed description). For saliva collection, Salivettes were used (Sarstedt, Nümbrecht, Germany). Directly after each scanning session, samples were centrifuged for 15 min with 2500 n/min and stored at -20°C . Laboratory analysis of the samples was done at the clinical laboratory of the Max

Planck Institute for Psychiatry Munich, Germany, by using the electrochemiluminescence immunoassay ECLIA on a cobas e 601 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). Intra- and inter-assay coefficients of variability were below 10% and 15%, respectively.

At each sampling time point, participants were requested to report their current stress level ("How stressed do you feel right now?") on a 10-point Likert scale, ranging from 1 = very low stress to 10 = very high stress.

During the stress task and each resting-state scan, heart rate was assessed with an infrared pulse oximeter (sampling rate of 50 Hz) placed on the index finger of the non-dominant hand. MATLAB R2012a (The Mathworks Inc.) was used to extract peak-to-peak intervals between heartbeats. Heart rate data were controlled for misdetection and ectopic beats with physiological noise modelling (Brooks et al., 2008). Finally, heart rate variance was assessed with KUBIOS HRV (Tarvainen et al., 2014).

Salivary cortisol concentrations, subjective stress ratings, and heart rate (variability) were tested with a repeated-measures ANCOVA with Time as within-subjects factor, followed by post-hoc paired *t*-tests using IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp) to confirm success of stress induction. We repeated these tests including SLoss [MCTQ/diary] as continuous between-subjects factor to test for effects of sleep loss on the physiological and psychological variables.

2.5. Scanning procedure

Two resting-state fMRI scans, one before and one directly after the stress task (see Fig. 1), were acquired on a Siemens 3 T Magnetom Trio system with a 32-channel head coil (Siemens, Erlangen, Germany) with the following parameters: T2*-weighted gradient-echo echo-planar

imaging (EPI) sequence, echo time (TE): 25 ms, repetition time (TR): 1560 ms, flip angle: 64°, number of volumes: 308, field of view: 192 mm × 192 mm, number of axial slices: 28 (descending slice order), in-plane matrix size: 64 × 64, 3 mm isotropic voxels, inter-slice gap: .75 mm, scan duration: 8 min 4 s. During resting-state scans, participants were required to lie still, keep their eyes open, and focus on a fixation cross on the monitor. For registration to standard space, a high-resolution structural image of the whole brain was acquired with the following parameters: T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence, TE: 2.52 ms, TR: 1900 ms, flip angle: 9°, field of view: 256 mm × 256 mm × 192 mm, 1 mm isotropic voxels, parallel imaging technique: GRAPPA acceleration factor 2, scan duration: 4 min 24 s.

Of note, besides the stress task the scanning procedure included another task paradigm (delayed match-to-sample with emotional distraction; see Fig. 1). The results of both tasks will be published elsewhere.

2.6. fMRI data preprocessing and statistical analysis

Preprocessing of resting-state fMRI data was conducted with FSL version 5.0 (FMRIB Software Library, <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) (Smith et al., 2004) and contained the following steps: motion correction with MCFLIRT (Jenkinson et al., 2002), slice timing correction for the descending slice order, brain extraction with BET (Smith, 2002), spatial smoothing with a Gaussian Kernel of 6 mm FWHM, independent component analysis (ICA)-based removal of artifacts with ICA-AROMA (Pruim et al., 2015), and application of a temporal high-pass filter of 125 s (.008 Hz). For registration to standard space, resting-state volumes were co-registered to the T1 image using boundary-based registration (BBR; Greve and Fischl, 2009), including a fieldmap to take into account distortions due to local field inhomogeneity. Non-linear normalization of the T1 image to the 2 mm MNI standard space template (Montreal Neurological Institute, Quebec, Canada) was done using Advanced Normalization Tools (ANTs; Avants et al., 2011). Resting-state data were then normalized to MNI standard space, applying the registration matrices and warp images from the two previous registration steps.

A quality check was performed to exclude participants with a mean framewise displacement > .5, and with clearly visible acquisition artifacts on temporal signal-to-noise-ratio and temporal standard deviation images, which were calculated across all volumes of each resting-state scan.

A seed-based correlation approach (Fox and Raichle, 2007) was applied to assess amygdala resting-state functional connectivity before and after the stress manipulation. For the purpose of time course extraction, two binary masks of the seed regions (left and right amygdala) were created in MNI standard space by using the Harvard-Oxford subcortical structural atlas, as implemented in FSL, applying a threshold of 80% probability for the selected voxels to lie in the amygdala. Next, the averaged time courses of activation within the amygdala were extracted from the preprocessed resting-state data for each subject and each amygdala mask separately. The same approach was applied to extract averaged time courses for cerebrospinal fluid (CSF) and deep white matter (WM). Subsequently, a general linear model (GLM) was set up for each individual, each amygdala seed, and each time point (pre-/post-stress) separately, with the amygdala time course as regressor of interest, and time courses of CSF and WM as nuisance regressors. As a result, four functional connectivity maps (left/right amygdala, pre-/post-stress) were obtained per participant. Next, the difference in between pre- and post-stress functional connectivity was calculated for each participant and each amygdala seed using a fixed effects analysis. These difference maps were then entered into a higher-level analysis, entering either SLoss [MCTQ] or SLoss [diary] as predictor and age as covariate. The resulting *t*-statistical maps then underwent Threshold-Free Cluster Enhancement (TFCE; Smith and

Nichols, 2009), using the default parameter settings ($H = 2$, $E = 0.5$, $C = 6$), and significance testing was carried out with permutation testing (4,000 iterations), using the in-house developed TFCE_mediation software (Lett et al., 2017). In the latter step, a null distribution of random results was generated against which the empirical findings were tested, which resulted in statistical images that are family-wise error corrected for multiple comparisons at $p < .05$. Furthermore, a region of interest (ROI) analysis was performed for brain regions of the DMN, based on previous results from our group on amygdala functional connectivity after psychosocial stress (Veer et al., 2011). This mask was created using the Harvard Oxford (Sub-)Cortical Probability Atlas, not only selecting the medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC), and precuneus as main DMN regions, but also the anterior insular cortex (AI) and dorsal ACC as main SN regions, and additionally the dorsolateral prefrontal cortex (DLPFC) and the hippocampus. We applied no probability threshold to these regions, so that the mask was as unbiased as possible. For the ROI analysis, permutation testing was carried out with the same settings, but only for voxels falling within the ROI mask.

The amygdala seeds and ROI mask, as well as the corrected and uncorrected statistical images of our analysis can be found under: <http://neurovault.org/collections/4612>.

3. Results

3.1. Sleep loss

SLoss [MCTQ] varied between 0 and 7.5 h/week ($M = 2.38$; $SD = 2.04$; $n = 45$), while SLoss [diary] ranged from .21 to 9.8 h during the week prior to scanning ($M = 1.9$; $SD = 1.79$; $n = 45$). SLoss [MCTQ] and SLoss [diary] were weakly associated ($r_s = .27$, $p = .035$).

3.2. Physiological and psychological stress response

All cortisol concentrations and subjective stress ratings are listed in Table 2. Saliva sample quality was insufficient to assess cortisol levels for $n = 2$ at t1, $n = 2$ at t2, $n = 1$ at t4, $n = 3$ at t5, $n = 1$ at t6, and $n = 2$ at t7 ($n = 40$ with data on all 7 sample time points). The repeated-measures ANCOVA on cortisol concentrations across all seven sample time points revealed a within-subjects effect of Time ($F(2.976, 116.072) = 9.995$, $p < .001$, $\eta^2_{\text{partial}} = .204$). Post-hoc *t*-tests did show an expected increase of cortisol concentrations from t1 to t3 ($t(42) = -1.84$, $p = .036$, one-tailed), as well as decrease of cortisol concentrations from t1 to t7 ($t(40) = 5.15$, $p < .001$), t4 to t6 ($t(43) = 3.62$, $p < .001$), and t4 to t7 ($t(42) = 5.297$, $p < .001$) reflecting hormonal stress recovery on top of the typical diurnal decline, while there was no significant difference between cortisol concentrations at t1 and those at t2, t4, t5, and t6 (Fig. 2A).

The repeated-measures ANCOVA on the subjective stress ratings showed a main effect of Time ($F(2.601, 114.45) = 43.335$, $p < .001$, $\eta^2_{\text{partial}} = .496$). Paired-sample *t*-tests did reveal an increase between ratings at t2 (pre-stress) and t3 (post-stress) ($t(44) = 9.97$, $p < .001$), an increase between t2 and t4 (post resting-state-2) ($t(44) = 3.16$, $p = .003$), and a decrease between t3 and t4 ($t(44) = -8.96$, $p < .001$) (Fig. 2B).

Due to technical problems, heart rate was not acquired for $n = 2$ during resting-state 1, $n = 3$ during stress induction, and $n = 1$ during resting-state 2 ($n = 41$ with data on all three time points). Mean values for heart rate frequency were 65.36 bpm ($SD = 8.85$) during resting-state 1, 78.64 bpm ($SD = 12.66$) during stress induction, and 65.72 bpm ($SD = 9.63$) during resting-state 2. A significant main effect of Time was revealed ($F(1.139, 45.565) = 91.239$, $p < .001$, $\eta^2_{\text{partial}} = .695$), indicating a change of heart rate between experimental conditions. Heart rates were higher during stress induction compared to resting-state 1 ($t(40) = -9.18$, $p < .001$), and lower during resting-state 2 compared to stress induction ($t(41) = 11.03$, $p < .001$).

Fig. 2. Mean levels of (A) saliva cortisol concentration (nmol/L) and (B) of subjective stress ratings from 1 (very low stress) to 10 (very high stress) plotted against the time course of the experiment: t1 = pre-scan, t2 = pre-stress, t3 = post-stress, t4 = post-resting-state 2, t5-t7 = after further task and debriefing. Time (t, min) is relative to the onset of stress induction. * $p < .05$, ** $p < .01$, *** $p < .001$. Error bars represent the standard errors of the mean.

Log-transformed heart rate variability ($n = 41$) averaged at 6.28 ($SD = .82$) during resting-state 1, 6.23 ($SD = .99$) during stress induction, and 6.32 ($SD = .88$) during resting-state 2. No main effect of Time was found ($F(1.611, 64.437) = .45$, $p = .597$, $\eta^2_{partial} = .011$).

Taken together, we consider the stress manipulation successful as cortisol levels and subjective stress ratings were increased after stress, and heart rate frequency was higher during stress. No associations of cortisol, heart rate, and stress ratings were found with sleep loss (both MCTQ and sleep log), neither on the repeated measures, nor on the post-hoc comparisons between time points.

3.3. Amygdala resting-state functional connectivity

In our seed-based correlations across all participants and both resting-state scans, patterns of left and right amygdala resting-state functional connectivity (RSFC) for both hemispheres were similar to results reported in previous studies (e.g., Roy et al., 2009; Veer et al., 2011), and comprised parts of the prefrontal cortex (including orbito-frontal cortex; OFC), temporal poles, hippocampus, and parts of the brainstem (Fig. 3).

No main effect of time (pre- vs. post-stress) was found, neither for

RSFC of the left nor right amygdala. However, a negative correlation between SLoss [diary] and the temporal difference in left amygdala RSFC was found for several cortical regions (post-stress > pre-stress; $p < .05$, whole-brain TFCE-corrected for multiple comparisons), comprising the right OFC, right inferior frontal gyrus (IFG), right anterior insular cortex (AI), right frontal pole, and right paracingulate gyrus (see Table 1). That is, participants with higher amounts of SLoss [diary] showed a decrease in amygdala functional connectivity with these brain regions after stress.

Masking for regions of the DMN as a priori regions of interest, additional SLoss [diary]-related decreased connectivity was found with the right superior frontal gyrus, right and left PCC, right middle frontal gyrus, and left dorsal anterior cingulate cortex (dACC) ($p < .05$, TFCE-corrected for multiple comparisons inside the ROI mask; see Fig. 4 and Table 1).

No association was found between SLoss [MCTQ] and amygdala RSFC, neither whole-brain nor when masking for our ROIs.

Of note, in our SLoss [diary]-based sleep loss data there were three outliers reporting 9.8, 6.45, and 5.68 h of sleep loss one week prior to scanning, although these still constitute a relevant phenotype in relation to our research question. However, when excluding these

Fig. 3. Main effects of seed-based correlation across all participants and both resting-state scans for left (A) and right (B) amygdala (t-values, thresholded between 10 and 15, $p < .05$, TFCE-corrected for multiple comparisons). R, right; L, left.

Table 1

Peak voxels and corresponding t-values of the negative association between SLoss [diary] and temporal difference of left amygdala RSFC. Effects in all regions listed are TFCE-corrected for multiple comparisons ($p < .05$).

Atlas region	Hemi-sphere	Cluster size 2 mm voxels	Peak voxel coordinates (MNI)			t-Value
			x	y	z	
Whole-brain analysis						
Orbitofrontal cortex, inferior frontal gyrus, anterior insular cortex	R	572	40	26	−8	4.34
Frontal pole	R	79	16	48	38	3.96
Paracingulate gyrus	R	44	6	32	40	3.88
ROI analysis						
Frontal pole, superior frontal gyrus, paracingulate gyrus	R	892	4	60	26	3.97
Posterior cingulate cortex	L/R	312	4	−18	42	4.17
Orbitofrontal cortex, inferior frontal gyrus	R	246	40	28	−8	4.30
Posterior cingulate cortex	R	197	12	−46	30	4.45
Middle frontal gyrus	R	154	42	28	40	4.00
Paracingulate gyrus	R	122	12	46	−2	4.03
Dorsal anterior cingulate cortex	L	96	−6	28	16	4.03
Frontal pole	L	8	−8	44	38	3.51

participants from our analyses, the whole-brain analysis remained significant for left amygdala RSFC to the IFG, the anterior insula and upper parts of the OFC, whereas the effects in regions included in our ROI analysis became non-significant. However, at a more lenient uncorrected level ($p < .001$), our ROI analysis without the outliers did reveal two clusters (right paracingulate gyrus, right OFC) that were also part of the initial ROI finding.

4. Discussion

Sleep loss, which could be considered a potent stressor in itself, affects quality of life in individuals worldwide. Although it is known that both stress and sleep loss affect activation and functional connectivity of brain regions supporting emotion processing and regulation, it is unclear how sleep loss affects stressor processing. Therefore, we aimed to investigate whether recent sleep loss could be associated with functional connectivity changes after induction of psychosocial stress.

In our study, we used two different measures of cumulative sleep loss. Sloss [MCTQ], on the one hand, was attained during recruitment with the Munich Chronotype Questionnaire (MCTQ) and should reflect chronic or more trait-like patterns of sleep behavior, as participants are asked for a subjective estimation of their general sleeping habits. Sloss [diary], on the other hand, was attained by calculating the same MCTQ parameters from the entries in a sleep diary of the week preceding scanning. Thus, sloss [diary] should represent a more state-like or acute 'snapshot' of participants' sleeping behavior, although to a certain extent this estimation could of course be driven by habitual sleeping patterns. Support for the latter may be found in the weak association between sloss [diary] and sloss [MCTQ] ($r_s = .27$, $p = .035$), as stated in our results section. As such, sloss [diary] should perhaps not be characterized as merely acute, but rather as a subacute type of sleep loss.

Our measures of the physiological and psychological stress response are in line with previous results on the effects of stress induction during the ScanSTRESS paradigm (Streit et al., 2014). Participants in our study showed a change of subjective stress ratings, heart rate, and cortisol along the stress manipulation: They felt more stressed directly after stress induction than directly before, showed higher heart rates during

than before and after the stress induction, and demonstrates a slight cortisol increase from baseline to the sampling time point after stress, which is all indicative of a successful stress manipulation. Furthermore, we did not find any associations of cortisol, heart rate, and stress ratings with sleep loss (both MCTQ and sleep diary), which is consistent with another recent finding where one night of total sleep deprivation had no effect on psychosocial stress reactivity of cortisol, salivary alpha amylase, subjective stress experience, and heart rate variability both in younger and older healthy individuals (Schwarz et al., 2018).

We indeed found a negative association of sleep loss (SLoss [diary]) with the stress-induced change of left amygdala RSFC to a variety of cortical brain regions, including the medial prefrontal cortex (MPFC), dorsolateral prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (dACC), anterior insula (AI), and posterior cingulate cortex (PCC). That is, compared to the pre-stress condition, the higher SLoss [diary] reported by participants, the more they showed a decrease in left amygdala RSFC with these regions after stress.

Our results are in line with previous findings regarding the impact of acute and chronic sleep deprivation on brain regions linked to affect processing and regulation (Krause et al., 2017). Yoo et al. (2007) demonstrated reduced amygdala functional connectivity to the MPFC for aversive visual stimuli after 35 h of total sleep deprivation. In our study, we found a similar pattern of reduced amygdala RSFC to the MPFC, though instead of aversive stimuli we conducted a stress-exposure procedure. Also, in two more recent studies (Lei et al., 2015; Shao et al., 2014) decreased amygdala RSFC to the PCC and DLPFC was shown after 36 h of total sleep deprivation. We found reduced left amygdala RSFC to the same regions, however in interaction with stress exposure. Furthermore, 24 h of total sleep deprivation have been shown to alter threat detection sensitivity of the central nodes of the salience network (SN) consisting of the amygdala, the dACC and the AI (Goldstein-Piekarski et al., 2015). In our study, we observed reduced left amygdala RSFC to the left dACC, as well as to the right AI. Thus, our findings suggest a stress-induced involvement of the same central SN nodes that are affected in the context of emotional discrimination after sleep deprivation.

Moreover, our results may shed light on aspects of neural adaptation stress in conjunction with naturally occurring sleep deprivation. It has been found that, under non-sleep-deprived circumstances, the

Table 2

Mean values and standard deviation (SD) of saliva cortisol concentrations (nmol/L) and subjective stress ratings (10-point Likert scale).

	n	t1 (−35)	t2 (0)	t3 (+20)	t4 (+35)	t5 (+50)	t6 (+70)	t7 (+100)
Cortisol, nmol/L (SD)	40	3.52 (1.69)	3.97 (2.59)	4 (2.81)	3.93 (2.84)	3.74 (2.53)	3.12 (1.99)	2.53 (1.16)
Stress ratings (SD)	41	2.88 (1.66)	2.85 (1.68)	5.41 (1.32)	3.46 (1.25)	4.22 (1.46)	2.83 (1.55)	2.37 (1.44)

Fig. 4. (A) Negative association between SLoss [diary] and temporal difference in left amygdala resting-state functional connectivity (RSFC; resting-state 2 > resting-state 1, t-values) with cortical midline structures ($p < .05$, TFCE-corrected for multiple comparisons inside the ROI mask). R = right; L = left. (B) Scatter plot illustrating the association between SLoss [diary] and temporal difference in mean left amygdala RSFC with the prefrontal activation patterns marked in (A), representing the largest cluster in the ROI analysis.

amygdala demonstrated increased RSFC to the MPFC and PCC one hour after stress (Veer et al., 2011) and to the precuneus directly after stress (Maron-Katz et al., 2016). This process was discussed as potentially reflecting an enhancement of neural regulatory feedback, memory processing of emotionally salient events, and self-evaluation due to emotional experience in the direct and prolonged aftermath of social stress. Interestingly, a different type of social stressor (watching aversive movie scenes) also yielded an increase of amygdala RSFC to the main hubs of the SN (dACC, AI), which was interpreted as a state of extended vigilance in the direct aftermath of moderate social stress (van Marle et al., 2010).

With regard to our own results, this putative neural adaptation mechanism seems to be influenced by sleep loss, even if only mild. Not only was our stress induction paradigm associated with a decrease of left amygdala RSFC to the two main nodes of the DMN (MPFC and PCC) in relation to the amount of sleep loss experienced in the week before scanning, we also identified a similar pattern of amygdala RSFC reduction to central parts of the SN (dACC and AI). Thus, naturally occurring sleep loss could be held responsible to inversely modulate and thereby disengage physiological patterns of early neural stress recovery within two important neurocognitive subsystems. However, future research is needed to further differentiate between the observed changes of amygdala RSFC to the DMN on the one hand and the SN on the other, as each might be associated with different neurobehavioral consequences.

Sleep loss dosage-dependent stress vulnerability due to impaired neural adaptation to stress also corroborates the link between psychopathology and sleep deprivation, which is well documented on an epidemiological basis (Baglioni et al., 2016). For instance, major depressive disorder has been linked to reduced amygdala RSFC to the VMPFC, dACC and AI (Veer et al., 2010). The similarity to the RSFC patterns found in our study may therefore advance our knowledge on why individuals suffering from insomnia have a more than two-fold risk to develop a major depression (Baglioni et al., 2011). Interestingly, a potential pathophysiological background for mood and anxiety disorders has recently been identified in terms of disrupted REM sleep patterns, which were found to be associated with maladaptive emotional processing and are generally known to reflect insufficient locus coeruleus silencing (Wassing et al., 2019).

4.1. Limitations

Although we obtained significant results when using the SLoss [diary] values, this was not the case when using the values from SLoss [MCTQ]. This difference, together with the rather weak association between our two measures of sleep loss, suggests there may exist more state- and trait-like effects of sleep loss. Data from sleep diaries might represent a rather state-like variable, since these were estimated during the seven days prior to our scanning session. The MCTQ, in contrast, asks for habitual sleep and waking times, which could mean that sleep timings during the week preceding scanning might be more influential than estimates of general sleeping patterns.

Second, our sleep loss parameters were exclusively acquired through subjective assessment methods. In the current study we did not use more objective techniques, like actigraphy or polysomnography, which could have provided more precise parameters of sleep on- and offset (Jungquist et al., 2015). Also, as we did not apply any electroencephalographic measurement, no statements can be made regarding our participants' sleep architecture and its potential influence on differential patterns of amygdala RSFC, which could be a valuable research point for future directions (Goldstein and Walker, 2014). For instance, an important focus could lie on dysfunctional REM sleep architecture, as it has recently been hypothesized to increase amygdala reactivity towards induction of aversive emotions (Wassing et al., 2019).

Third, to maximize wakefulness, we conducted an eyes-open resting-state procedure. However, we cannot rule out the possibility that participants with more sleep loss experienced more drowsiness during the second resting-state scan, which could at least partly have influenced the differences in connectivity independently of stress.

Fourth, different times of scanning sessions might have led to different outcomes. Since our sessions took place on Wednesday and Thursday evenings, we presumably detected lower amounts of cumulative sleep loss than would have been the case if scanning had been scheduled on Fridays. Nonetheless, even the normal variation in sleep loss in our otherwise healthy individuals was associated with changes in functional connectivity after social stress.

Fifth, we identified three outliers in our SLoss [diary] data. One extreme outlier reported 9.8 h of cumulative sleep loss over one week prior to scanning, whereas two others reported 6.45 and 5.68 h of sleep loss. Still, we did not want to exclude these outliers, as these in fact

reflect meaningful variance on the natural spectrum of our sleep loss data, which most likely would have been more continuous with a bigger sample. This is supported by the notion that our ROI analysis without the outliers, albeit only at an uncorrected threshold, showed two clusters (right paracingulate gyrus, right OFC) that were also part of the initial ROI finding. This indicates that removing the extreme values also narrowed the variance in sleep loss by eliminating the ones that most likely would show an effect at all. However, replication is needed to show that our findings are not coincidental.

Sixth, generalizability to the general population is limited by having included male participants only. Because women on average demonstrate weaker cortisol responses to psychosocial stress than men (Kirschbaum et al., 1992), which is furthermore influenced by menstrual phase at the time of testing and use of anticonceptives (Dedovic et al., 2009), it was decided to create an as homogeneous sample as possible given the overall moderate sample size.

Seventh, potential influence of the so-called ‘wake maintenance zone’ (WMZ) on our results should be taken into consideration. WMZ refers to a phenomenon that, due to sleep pressure, cognitive capacities and arousal are increased instead of reduced during a two to three-hour window of the late evening right before onset of melatonin secretion (Shekleton et al., 2013). As we recruited participants with later chronotypes, they reported an average sleep onset time of 0:11 a.m. (± 59 min) on working days and of 1:38 am (± 82 min) on free days the week before scanning (sleep diaries). We therefore conclude that the majority of our participants should not have reached the WMZ during their participation in the experiment. However, we cannot completely rule out influences of this phenomenon and suggest that future replications should assess the onset of melatonin secretion in order to fully determine the influence of the WMZ in this context.

Eighth, it would be desirable to distinguish between effects of general diurnal patterns of cortisol decline and those of the actual stress recovery. Unfortunately, differentiating between the two effects would only be possible by comparing the slopes of decline with those from a non-stressed control group, which was not assessed in the current study.

Finally, although we discovered an interaction effect between sleep loss and temporal change of amygdala RSFC, we did not find a main effect of time when comparing pre- and post-stress connectivity irrespective of sleep loss. Although interaction effects in absence of main effects are statistically valid, this could also reflect a lack of power. However, as subjective stress, heart rate, and cortisol did increase due to the stress manipulation, a change in connectivity by the stressor alone should perhaps have been more evident in our data rather than its interaction with sleep loss. Therefore, replication is warranted to assess the robustness of this interaction.

4.2. Conclusions

In this study, we examined the association between sleep loss and amygdala RSFC changes in response to psychosocial stress. Taken together, stronger reduction of left amygdala RSFC from the pre- to post-stress acquisition with central nodes of the DMN (MPFC, PCC) and SN (dACC, AI), as well as to the DLPFC, was associated with higher sleep loss. Our findings are opposite to the effects found in other studies under non-sleep-deprived conditions in the direct and prolonged aftermath of stress. This might suggest that neural stress adaptation mechanisms are potentially compromised when encountering higher amounts of sleep loss. As our results demonstrate similarity to those found in affective disorders, such as major depressive disorder, our findings may not only contribute to effects of sleep loss in everyday life of healthy individuals, but also help in explaining the high prevalence of sleep deficiencies among patients with depression and other mental disorders.

Declaration of Competing Interest

Servier Deutschland GmbH kindly provided funding for the study, but had no involvement in study design, data acquisition and analysis, interpretation of the results, and drafting the manuscript.

Author contributions

AD, IV, HW, and MA designed the study. JN, AD, and IV acquired the data. JN, AD and IV analyzed the data. The manuscript was written by JN, AD, and IV, and amended and approved by all co-authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.psyneuen.2020.104585>.

References

- Avants, B.B., Tustison, N.J., Song, G., Cook, P.A., Klein, A., Gee, J.C., 2011. A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* 54, 2033–2044.
- Baglioni, C., Battagliese, G., Feige, B., Spiegelhalter, K., Nissen, C., Voderholzer, U., Lombardo, C., Riemann, D., 2011. Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. *J. Affect. Disord.* 135, 10–19.
- Baglioni, C., Nanovska, S., Regen, W., Spiegelhalter, K., Feige, B., Nissen, C., Reynolds, C.F., Riemann, D., 2016. Sleep and mental disorders: a meta-analysis of polysomnographic research. *Psychol. Bull.* 142, 969–990.
- Brooks, J.C., Beckmann, C.F., Miller, K.L., Wise, R.G., Porro, C.A., Tracey, I., Jenkinson, M., 2008. Physiological noise modelling for spinal functional magnetic resonance imaging studies. *Neuroimage* 39, 680–692.
- Brower, K.J., Aldrich, M.S., Robinson, E.A., Zucker, R.A., Greden, J.F., 2001. Insomnia, self-medication, and relapse to alcoholism. *Am. J. Psychiatry* 158, 399–404.
- Chuah, L.Y., Dolcos, F., Chen, A.K., Zheng, H., Parimal, S., Chee, M.W., 2010. Sleep deprivation and interference by emotional distracters. *Sleep* 33, 1305–1313.
- Costafreda, S.G., Brammer, M.J., David, A.S., Fu, C.H., 2008. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies. *Brain Res. Rev.* 58, 57–70.
- Dedovic, K., Wadiwalla, M., Engert, V., Pruessner, J.C., 2009. The role of sex and gender socialization in stress reactivity. *Dev. Psychol.* 45, 45–55.
- Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.* 130, 355–391.
- Duchene, A., Tessler, E., Dedovic, K., Engert, V., Pruessner, J.C., 2012. Effects of panel sex composition on the physiological stress responses to psychosocial stress in healthy young men and women. *Biol. Psychol.* 89, 99–106.
- Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci.* 8, 700–711.
- Germain, A., 2013. Sleep disturbances as the hallmark of PTSD: where are we now? *Am. J. Psychiatry* 170, 372–382.
- Goldstein, A.N., Greer, S.M., Saletin, J.M., Harvey, A.G., Nitschke, J.B., Walker, M.P., 2013. Tired and apprehensive: anxiety amplifies the impact of sleep loss on aversive brain anticipation. *J. Neurosci.* 33, 10607–10615.
- Goldstein, A.N., Walker, M.P., 2014. The role of sleep in emotional brain function. *Annu. Rev. Clin. Psychol.* 10, 679–708.
- Goldstein-Piekarski, A.N., Greer, S.M., Saletin, J.M., Walker, M.P., 2015. Sleep deprivation impairs the human central and peripheral nervous system discrimination of social threat. *J. Neurosci.* 35, 10135–10145.
- Greve, D.N., Fischl, B., 2009. Accurate and robust brain image alignment using boundary-based registration. *Neuroimage* 48, 63–72.
- Gujar, N., Yoo, S.S., Hu, P., Walker, M.P., 2011. Sleep deprivation amplifies reactivity of brain reward networks, biasing the appraisal of positive emotional experiences. *J. Neurosci.* 31, 4466–4474.
- Hasler, B.P., Kirisci, L., Clark, D.B., 2016. Restless sleep and variable sleep timing during late childhood accelerate the onset of alcohol and other drug involvement. *J. Stud. Alcohol Drugs* 77, 649–655.
- Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17, 825–841.
- Jungquist, C.R., Pender, J.J., Klingman, K.J., Mund, J., 2015. Validation of capturing sleep diary data via a wrist-worn device. *Sleep Disord.* 2015, 758937.

- Kecklund, G., Axelsson, J., 2016. Health consequences of shift work and insufficient sleep. *BMJ* 355, i5210.
- Killgore, W.D., 2013. Self-reported sleep correlates with prefrontal-amygdala functional connectivity and emotional functioning. *Sleep* 36, 1597–1608.
- Kirschbaum, C., Wüst, S., Hellhammer, D., 1992. Consistent sex differences in cortisol responses to psychological stress. *Psychosom. Med.* 54, 648–657.
- Krause, A.J., Simon, E.B., Mander, B.A., Greer, S.M., Saletin, J.M., Goldstein-Piekarski, A.N., Walker, M.P., 2017. The sleep-deprived human brain. *Nat. Rev. Neurosci.* 18, 404–418.
- LeDoux, J., 2003. The emotional brain, fear, and the amygdala. *Cell. Mol. Neurobiol.* 23, 727–738.
- Lei, Y., Shao, Y., Wang, L., Ye, E., Jin, X., Zou, F., Zhai, T., Li, W., Yang, Z., 2015. Altered superficial amygdala-cortical functional link in resting state after 36 hours of total sleep deprivation. *J. Neurosci. Res.* 93, 1795–1803.
- Lett, T.A., Waller, L., Tost, H., Veer, I.M., Nazeri, A., Erk, S., Brandl, E.J., Charlet, K., Beck, A., Vollstädt-Klein, S., Jorde, A., Kiefer, F., Heinz, A., Meyer-Lindenberg, A., Chakravarty, M.M., Walter, H., 2017. Cortical surface-based threshold-free cluster enhancement and cortexwise mediation. *Hum. Brain Mapp.* 38, 2795–2807.
- Maron-Katz, A., Vaisvaser, S., Lin, T., Hendler, T., Shamir, R., 2016. A large-scale perspective on stress-induced alterations in resting-state networks. *Sci. Rep.* 6, 21503.
- Motomura, Y., Kitamura, S., Oba, K., Terasawa, Y., Enomoto, M., Katayose, Y., Hida, A., Moriguchi, Y., Higuchi, S., Mishima, K., 2013. Sleep debt elicits negative emotional reaction through diminished amygdala-anterior cingulate functional connectivity. *PLoS One* 8, e56578.
- Oei, N.Y., Veer, I.M., Wolf, O.T., Spinhoven, P., Rombouts, S.A., Elzinga, B.M., 2012. Stress shifts brain activation towards ventral 'affective' areas during emotional distraction. *Soc. Cogn. Affect. Neurosci.* 7, 403–412.
- Prather, A.A., Bogdan, R., Hariri, A.R., 2013. Impact of sleep quality on amygdala reactivity, negative affect, and perceived stress. *Psychosom. Med.* 75, 350–358.
- Pruim, R.H., Mennes, M., van Rooij, D., Llera, A., Buitelaar, J.K., Beckmann, C.F., 2015. ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage* 112, 267–277.
- Roenneberg, T., Kuehne, T., Pramstaller, P.P., Ricken, J., Havel, M., Guth, A., Mellow, M., 2004. A marker for the end of adolescence. *Curr. Biol.* 14, R1038–R1039.
- Roy, A.K., Shehzad, Z., Margulies, D.S., Kelly, A.M., Uddin, L.Q., Gotimer, K., Biswal, B.B., Castellanos, F.X., Milham, M.P., 2009. Functional connectivity of the human amygdala using resting state fMRI. *Neuroimage* 45, 614–626.
- Schwarz, J., Gerhardsson, A., van Leeuwen, W., Lekander, M., Ericson, M., Fischer, H., Kecklund, G., Åkerstedt, T., 2018. Does sleep deprivation increase the vulnerability to acute psychosocial stress in young and older adults? *Psychoneuroendocrinology* 96, 155–165.
- Shao, Y., Lei, Y., Wang, L., Zhai, T., Jin, X., Ni, W., Yang, Y., Tan, S., Wen, B., Ye, E., Yang, Z., 2014. Altered resting-state amygdala functional connectivity after 36 hours of total sleep deprivation. *PLoS One* 9, e112222.
- Shekleton, J.A., Rajaratnam, S.M., Gooley, J.J., Van Reen, E., Czeisler, C.A., Lockley, S.W., 2013. Improved neurobehavioral performance during the wake maintenance zone. *J. Clin. Sleep Med.* 9, 353–362.
- Smith, S.M., 2002. Fast robust automated brain extraction. *Hum. Brain Mapp.* 17, 143–155.
- Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 44, 83–98.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23 (Suppl. 1), S208–S219.
- Streit, F., Haddad, L., Paul, T., Frank, J., Schäfer, A., Nikitopoulos, J., Akdeniz, C., Lederbogen, F., Treutlein, J., Witt, S., Meyer-Lindenberg, A., Rietschel, M., Kirsch, P., Wüst, S., 2014. A functional variant in the neuropeptide S receptor 1 gene moderates the influence of urban upbringing on stress processing in the amygdala. *Stress* 17, 352–361.
- Tarvainen, M.P., Niskanen, J.P., Lipponen, J.A., Ranta-Aho, P.O., Karjalainen, P.A., 2014. Kubios HRV—heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220.
- Vaisvaser, S., Lin, T., Admon, R., Podlipsky, I., Greenman, Y., Stern, N., Fruchter, E., Wald, I., Pine, D.S., Tarrasch, R., Bar-Haim, Y., Hendler, T., 2013. Neural traces of stress: cortisol related sustained enhancement of amygdala-hippocampal functional connectivity. *Front. Hum. Neurosci.* 7, 313.
- van Marle, H.J., Hermans, E.J., Qin, S., Fernández, G., 2010. Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. *Neuroimage* 53, 348–354.
- Veer, I.M., Beckmann, C.F., van Tol, M.J., Ferrarini, L., Milles, J., Veltman, D.J., Aleman, A., van Buchem, M.A., van der Wee, N.J., Rombouts, S.A., 2010. Whole brain resting-state analysis reveals decreased functional connectivity in major depression. *Front. Syst. Neurosci.* 4.
- Veer, I.M., Oei, N.Y., Spinhoven, P., van Buchem, M.A., Elzinga, B.M., Rombouts, S.A., 2011. Beyond acute social stress: increased functional connectivity between amygdala and cortical midline structures. *Neuroimage* 57, 1534–1541.
- Wassing, R., Lakbila-Kamal, O., Ramautar, J.R., Stoffers, D., Schalkwijk, F., Van Someren, E.J.W., 2019. Restless REM sleep impedes overnight amygdala adaptation. *Curr. Biol.* 29 2351–2358.e2354.
- Wittchen, H.-U., Zaudig, M., Fydrich, T., 1997. *Strukturiertes Klinisches Interview für DSM-IV. Hogrefe, Göttingen.*
- Yoo, S.S., Gujar, N., Hu, P., Jolesz, F.A., Walker, M.P., 2007. The human emotional brain without sleep—a prefrontal amygdala disconnect. *Curr. Biol.* 17, R877–878.