

Sleep deprivation affects fear memory consolidation: bi-stable amygdala connectivity with insula and ventromedial prefrontal cortex

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Abstract

Sleep plays an important role for successful fear memory consolidation. Growing evidence suggests that sleep disturbances might contribute to the development and the maintenance of posttraumatic stress disorder (PTSD), a disorders characterized by dysregulations in fear learning mechanisms, as well as exaggerated arousal and salience processing. Against this background, the present study examined the effects of sleep deprivation (SD) on the acquisition of fear and the subsequent neural consolidation. To this end, the present study assessed fear acquisition and associated changes in fMRI-based amygdala-functional connectivity following 24 h of SD. Relative to non-sleep deprived controls, SD subjects demonstrated increased fear ratings and skin conductance responses (SCR) during fear acquisition. During fear consolidation SD inhibited increased amygdala-ventromedial prefrontal cortex (vmPFC) connectivity and concomitantly increased changes in amygdala-insula connectivity. Importantly, whereas in controls fear indices during acquisition were negatively associated with amygdala-vmPFC connectivity during consolidation, fear indices were positively associated with amygdala-insula coupling following SD. Together the findings suggest that SD may interfere with vmPFC control of the amygdala and increase bottom-up arousal signaling in the amygdala-insula pathway during fear consolidation, which might mediate the negative impact of sleep disturbances on PTSD symptomatology.

Key words: sleep deprivation; fear consolidation; amygdala; insula; vmPFC

Introduction

Sleep plays an important role for successful fear memory consolidation, promoting the discrimination between fear-relevant and safety cues (Melo and Ehrlich, 2016; Menz *et al.*, 2016). In a clinical context, trauma-induced or predated sleep disturbances have been proposed as etiological factor for the development of posttraumatic stress disorder (PTSD) (Pace-Schott *et al.*, 2015). PTSD is an anxiety disorder that may develop after the

experience of threatening situations. On the symptomatic level, the disorder is characterized by persistent re-experiencing of the threatening situation, avoidance, hyperarousal and sleep disturbances, i.e. insomnia and nightmares (Risbrough, 2015; Yehuda and LeDoux, 2007).

Neurobiological models propose that disruptions in the domains of fear learning, particularly a failure to extinguish the conditioned fear response and exaggerated salience of

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the threatening stimulus, play an important role in the development and maintenance of anxiety disorders (VanElzakker et al., 2014). With respect to PTSD, alterations during the stage of fear acquisition and fear memory consolidation have been directly associated with the severity of the initial PTSD symptomatology (Liberzon and Abelson, 2016; Zuj et al., 2016).

Animal models and human data suggest that a 'fear network', incorporating amygdala, insula, anterior cingulate cortex, and hippocampus, plays a pivotal role in the acquisition of fear (Etkin et al., 2011; Etkin and Wager, 2007; Fullana et al., 2016; Greco and Liberzon, 2016; Hartley et al., 2011; LaBar and Cabeza, 2006). Neurobiological studies examining the fear consolidation process indicate a critical role of the amygdala, hippocampal formation, insula, and circuits connecting the amygdala with medial frontal regions, including the dorsomedial prefrontal cortex (dmPFC), dorsal anterior cingulate cortex (dACC), and medial prefrontal cortex (mPFC) in fear memory consolidation. Moreover, previous studies observed an increase in functional connectivity between the amygdala and several other brain regions including the hippocampus and medial prefrontal cortex 24 h following the acquisition, indicating that these changes in the interaction of the brain regions reflect the consolidation of fear (Schultz, 2014). Specifically, Van Marle et al. found that increased stress levels (assessed by heart rate, salivary cortisol and negative affect) concomitantly increased amygdala-dACC and amygdala-insula resting state functional connectivity (RSFC) following experimentally-induced, moderate psychological stress (Van Marle et al., 2010). Furthermore, during the consolidation window following fear acquisition, activity in the parahippocampus, insula, thalamus and vmPFC was enhanced, with findings on associations between resting state activity in the vmPFC during consolidation and subjective fear ratings during the preceding acquisition stage pointing to a key role of the vmPFC in fear consolidation (Feng et al., 2013). On the network level, connectivity of the limbic core nodes, particularly increased connectivity of the amygdala-dACC and hippocampal-insula pathway, as well as decreased amygdala-mPFC has been implicated in fear consolidation, with findings on associations between fear indices and amygdala-vmPFC coupling further emphasizing the importance of this pathways (Feng et al., 2014).

Experimentally induced SD has been associated with altered cognitive and emotional functioning, including impaired executive control, working memory, and attention (Anderson and Platten, 2011; Ma et al., 2015; Scott et al., 2006), as well as increased negative emotionality which has been associated with impaired interaction between the amygdala and medial prefrontal regions suggesting deficient prefrontal-top down control of amygdala emotional reactivity (Ben Simon et al., 2017; Lei et al., 2015; Motomura et al., 2013).

Functional MRI studies suggest that SD-induced deficits in regulatory control during negative aversive stimuli may stem from SD-induced decreases in amygdala-vmPFC connectivity coupled with increased functional connectivity of amygdala with autonomic brainstem regions (Yoo et al., 2007). Further evidence for reduced prefrontal-amygdala top-down control comes from studies reporting SD decreased functional connectivity between subregions of the amygdala, particularly the basolateral and the superficial subregions engaged in fear reactivity, with medial frontal/dorsal cingulate executive control regions, in the context of enhanced functional connectivity with emotion processing regions such as the precuneus and the parahippocampus (Lei et al., 2015; Yoo et al., 2007; Yu-Feng et al., 2007). However, increased amygdala functional connectivity with other regions of the prefrontal control network, including the

rostral ACC and medial PFC (Lei et al., 2015; Shao et al., 2014) has also been reported following SD, suggesting differential effects on the amygdala-prefrontal circuitries. A number of studies suggested that SD may lead to heightened noradrenergic tone promoting an exaggerated and over-generalized hyperactivity in the core nodes of the affective salience networks, including the amygdala, insula, and dorsal ACC (Franzen et al., 2009; Goldstein et al., 2013; Goldstein and Walker, 2014). Specifically, SD has been shown to amplify preemptive anterior insula responses during the anticipation of potentially aversive experiences, with findings on associations between activity in the insula and trait anxiety emphasizing the key role of the insula in the anticipation of threat via monitoring internal interoceptive states (Goldstein et al., 2013). Furthermore, SD impaired interoceptive signaling-based discrimination of threatening from affiliative social cues in the amygdala and the insula, with findings on associations between REM gamma activity and insula discrimination ability emphasizing the link between sleep and insula affective processing (Goldstein-Piekarski et al., 2015). Despite accumulating evidence for SD-induced changes in the pathways underlying fear consolidation and salience processing and the proposed importance of sleep disturbances in PTSD, effects of SD on fear consolidation processes in these pathways have not been systematically examined.

Resting state functional connectivity (RSFC) has been shown an effective method to examine the functional interplay between brain regions in the absence of external stimulation (subjects are usually asked to rest and think of nothing in particular) (Andrews-Hanna et al., 2007; Greicius et al., 2009; Hagmann et al., 2008; Vincent et al., 2007), including fear acquisition associated changes during consolidation (Feng et al., 2014; Feng et al., 2016). Moreover, the method provides a strategy to evaluate the regulatory relationship between different brain regions (Banks et al., 2007; Burghy et al., 2012; Kim et al., 2011). To this end, the present study employed a RSFC approach to examine fear acquisition induced changes in the amygdala intrinsic networks to determine effects of SD on fear consolidation. In line with previous studies (Killgore, 2013), we examined whether associations between fear consolidation induced changes in amygdala RSFC and subjective and objective fear indices at the stage of fear acquisition vary as a function of SD.

Based on previous findings (Yoo et al., 2007), we expected that 24 h of SD would enhance subjective and objective indices of fear conditioning while disrupting functional connectivity between the amygdala and vmPFC, reflecting SD induced impaired top-down control capacity of the vmPFC. Moreover, we expected that SD would increase the connectivity of the amygdala with the insula, reflecting exaggerated salience and arousal towards the fear stimulus. Further, we expected that associations between subjective and objective measures of fear acquisition and subsequent consolidation-associated neural activity would vary as a function of SD. Finally, we assessed behavioral indices of fear reactivity at a later time point by testing subjective and objective response to conditioned stimulus (mean of all trials over extinction), and we predicted that SD would also enhance subjective and objective indices at fear extinction stage following 24 h sleep recovery.

Materials and methods

Participants

Seventy right-handed, non-smoking college students from the Southwest University (Chongqing, China) were recruited for the

present study. All participants abstained from caffeine, alcohol, physical activities, intense mental and novel activities in the before 72 h prior and during the entire period of the study. All participants had normal or corrected-to-normal vision and none had a history of, or a current psychiatric disorder, a neurological disease or a head injury. None of the subjects had experienced a jet lag during the month before the experiment. Participants were randomly assigned to the SD or the control group ($n=35$, control group; $n=35$, SD group) balanced for age and gender ($P=0.23$) (control group: $M_{age}=20.93$, $SD=1.59$ years, 16 females; SD group: $M_{age}=21.89$, $SD=1.97$, 11 females). Participants from the SD group arrived at the laboratory at 8:00AM and stayed awake 24h without napping. On the next day at 8:00AM, the participants underwent a baseline resting state acquisition (Rest 1, before fear conditioning), a fear conditioning paradigm followed immediately by a second resting state acquisition (Rest2, after fear conditioning). Participants in the control group had normal sleep before the experiment (slept before 11:00PM, duration >8h) and reported good sleep quality as assessed by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). Additionally, all participants reported generally good sleep habits (>8h of sleep/night; going to bed no later than 12:00 AM; getting up before 8:00 AM) as assessed by a two week sleep diary. To control for factors that might affect emotional processing participants underwent a thorough questionnaire based psychological assessment on day 1 [including assessments of anxiety, State Trait Anxiety Inventory, STAI, (Spielberger, 1983); depression, Self-Rating Depression Scale, SDS, (Zung, 1965), and mood, Positive and Negative Affect Scale, PANAS (Watson et al., 1988)]. Importantly, none of the surveys yielded group differences (Table 1). Written informed consent was obtained from all participants. The study and all procedures were approved by the Institutional Review Board of the Southwest University and the study was in accordance with the latest revision of the Declaration of Helsinki.

Additionally, examining mood that was assessed before and after SD (using the PANAS). For the positive affect ratings as assessed by the PANAS, a two-way mixed ANOVA analysis that included group (control group vs SD group) and time (day 1, day 2) revealed a significant interaction effect [$F(1, 68)=5.91$, $P<0.05$]. The simple effect analysis showed that there was no significant difference between the SD and control group on day1 [$t(68)=0.18$, $P=0.86$], but the control group had significantly higher positive ratings on day2 (before the experiment) than the SD group [$t(68)=2.90$, $P<0.005$]. For negative affect ratings, a corresponding two-way mixed ANOVA analysis revealed a significant interaction effect [$F(1, 68)=6.78$, $P<0.01$]. The simple effect analysis showed that there was no significant difference between SD group and control group on day1 [$t(68)=0.38$, $P=0.71$], but the SD group had higher negative ratings on day2

Table 1. The mean and standard deviation of SD group and control group on all scales

Scale	Control group		SD group		P
	M	SD	M	SD	
Positive emotion	27.70	5.54	27.07	7.02	0.65
Negative emotion	14.13	3.44	13.11	2.74	0.19
State anxiety	35.05	5.13	36.65	7.32	0.28
Trait anxiety	42.05	7.97	40.33	9.43	0.90
Depression	44.45	7.37	44.35	7.64	0.96
Sleep quality	4.35	1.73	4.07	1.78	0.47

(before experiment) compared to the control group [$t(68)=2.37$, $P<0.05$]. In line with these findings, another simple effect analysis showed that the positive emotion decreased following SD [$t(34)=5.70$, $P<0.001$], while the negative emotion increased following the SD [$t(34)=3.0$, $P<0.005$]. However, there was no significant difference between two times (day1 and day2) measurement for positive and negative affect respectively in the control group [positive emotion: [$t(34)=1.70$, $P=0.10$]; negative emotion: [$t(34)=0.48$, $P=0.63$]].

Design and procedure

Participants were randomly assigned to two experimental groups: the SD group and the control group. Participants in the SD group arrived at the laboratory at 8:00AM and stayed awake 24h without napping. On the next day at 8:00AM, the participants underwent the fear conditioning task and fMRI assessments (Rest1 and Rest2). Participants in the control group had normal sleep before the fear conditioning procedure and fMRI assessment (Rest1 and Rest2) (Figure 1A).

The detailed procedures were as follows: The experiment began with a baseline rest condition (Rest1, 8 min), followed by the fear acquisition paradigm and a subsequent resting state acquisition during fear consolidation (Rest2, 8 min) (Figure 1B). For the fear acquisition task, three square stimuli with different colors (blue, yellow or green) served as conditioned stimuli (CSa+, CSb+ and CS-), and a mild electric shock to the wrist (200 ms) coupled to 43.75% of the CSa+ and CSb+ (18CSa+, 18CSb+, 18CS-, 14CSa+ and 14CSb+ with shock) served as unconditioned stimulus (US), applied for a duration of 200 ms before the CS+ ended. For each participant, individual shock intensities (uncomfortable but not painful) were determined. With respect to the shock level (pain level, mA), shock intensities did not differ between the groups [SD group: $M=0.88$, $SD=0.31$; control group: $M=0.90$, $SD=0.57$; $t(68)=0.21$, $P=0.83$]. The CS (CSa+, CSb+ and CS-) were each presented in a pseudorandom order with 4 s duration and separated by a 6–10 s inter-trial interval. CS color was counterbalanced across the experimental groups. Participants were instructed to try to figure out the relationship between the color of the squares and the shock. During the resting state, participants were instructed to keep their eyes open and look at a fixation cross, let their mind wander, without falling asleep or moving. All participants

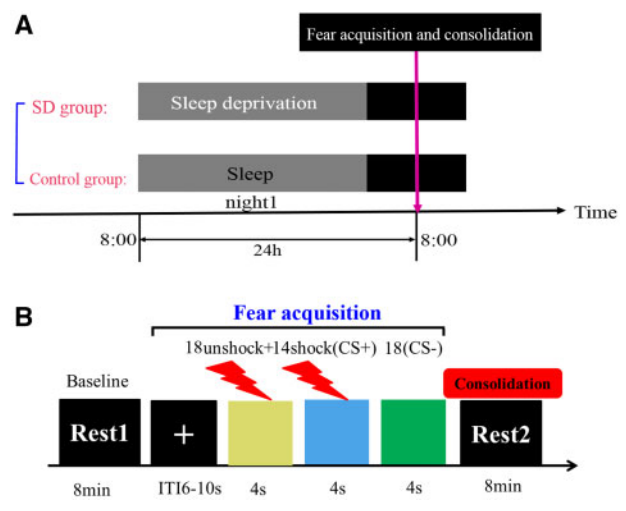


Fig. 1. (A, B) Experimental procedure.

included in the final analysis confirmed that they had not fallen asleep during the fMRI acquisition (Figure 1B).

Day3 consisted of reminder and extinction procedure to test the maintenance of the effects following normal sleep. During the reminder, the CSa+ was presented once (unreinforced), followed by a 10 min break. Following the break, extinction immediately followed for all participants and consisted of non-reinforced presentations of the three stimuli (21CSa+, 22CSb+, 22CS-). Given that the current manuscript focuses on the effects of fear acquisition rather than fear reminders, only results from the non-reminded CSb+ and the CS- during extinction will be reported in the present manuscript.

Psychophysiology assessment

Skin conductance responses were measured using shielded Ag-AgCl electrodes, which were connected to the BioPac System skin conductance module sampling at a rate of 200 HZ. Electrodes were attached to the second and the third finger of the left hand. In line with previous studies (Schiller et al., 2013; Schiller et al., 2010), the strengths of the SCR responses was defined as the difference between the maximum and minimum response amplitude in a time-window of 0.5 and 4.5 s after CS onset and a criterion for the smallest recordable SCR = 0.02 μ S. The raw SCR data were initially square-root-transformed to normalize the distribution. The normalized scores were scaled according to each subject's unconditioned response by dividing each response by the mean square-root-transformed unconditioned stimulus response. The exclusion criteria were based on the differential response to the CS+ and CS- at fear acquisition stage. That is, subjects were excluded if during fear acquisition the difference was in the opposite direction (CS->CSa+ or CS->CSb+). Moreover, we also added an additional criterion of equivalent fear acquisition to CSa+ and CSb+, which the subjects failed to show equivalent conditioned threat acquisition to the CSa+ and CSb+ (difference > 0.1 μ S). As a measure of subjective fear intensity subjective fear ratings of the stimuli (CSa+, CSb+ and CS-) were obtained immediately following the acquisition and the extinction stage using a 1-7 fearfulness scale (1: mildly; 4: moderately; and 7: extremely; each CS was presented four times during the rating).

Image acquisition and analysis

fMRI acquisition. Data were acquired on a Siemens 3 T MRI system (Siemens Magnetom Trio TIM, Erlangen, Germany). Head movement was restricted using foam cushions (>2 mm, 2 degree). Functional images were acquired using a T2*-weighted echo-planar image pulse sequence with the following parameters: 32 slices; 4 mm slice thickness; voxel size = 3.4 × 3.4 × 4 mm; TR = 2000 ms; TE = 25 ms; FOV = 220 × 220 mm²; matrix size = 64 × 64; flip angle = 85°. Additional, T1-weighted structural images were recorded to improve normalization (total of 176 slices at a thickness of 1 mm and in-plane resolution of 0.98 × 0.98 mm, TR = 1900 ms; TE = 2.52 ms; flip angle = 9°; FoV = 250 × 250 mm²).

fMRI data analysis. Resting-state fMRI data were analyzed using SPM12, DPABI2.1 and REST1.8 software packages (Chao-Gan and Yu-Feng, 2010; Friston et al., 1994; Song et al., 2011). The first five functional volumes were discarded to allow MRI equilibration. For the functional T2*-weighted images, slice timing was used to correct slice acquisition order, realigned was used to control motion effects and to estimate the six head motion parameters. For normalization, the T1-weighted structural images were

co-registered to the EPI mean images and segmented into white matter, gray matter, and Cerebrospinal fluid (CSF). The functional images were next normalized to MNI space using a 3 × 3 × 3 mm³ voxel resolution. The normalized data were spatially smoothed using a 6 mm the full width at half maximum (FWHM) kernel, and linear drift was removed and a band pass filter of 0.01–0.08 Hz was applied before the calculation of voxel wise and ROI wise indices for each subject. For the voxel wise analysis, the correlation was calculated between the amygdala seeds and the whole brain (brain activation map), whereas for the region of interest (ROI) wise analysis, a correlation analysis was performed between extracted time courses from the amygdala, insula and vmPFC, respectively (correlation coefficient) (Feng et al., 2014, 2016).

Regions of interest analysis

To determine effects of SD on fear consolidation associated amygdala networks, particularly pathways connecting the amygdala with the insula and vmPFC, we performed a voxel wise and a ROI analysis. For the voxel wise analysis, we performed a whole-brain voxel-based correlation using time courses obtained from left and right amygdala seeds (separately) with all voxels in the entire brain. We used the anatomical amygdala as seed region, defined by structural masks from the PickAtlas toolbox (Wake Forest University School of Medicine). The functional connectivity was estimated based on the detrended, filtered, and covariate-controlled data. Covariates included the six head motion parameters, white matter and CSF signal. Each participant's time courses were obtained separately from activation maps, and were then used as regressors in a voxel-based whole-brain correlation analysis. Importantly, the time course from all voxels from the anatomically defined amygdala was used as a regressor for each participant. Effects of SD were examined using two-way mixed ANOVA models with the between-subject factor group (SD vs controls) and the within-subject factor acquisition time (before vs after fear acquisition). Subsequent simple effects analyses were used to disentangle the effects, including paired t-test comparing RSFC before (Rest 1) and after fear acquisition (Rest 2) with the experimental groups. Group-level random effects analysis were performed using a height threshold of $P < 0.001$ and a cluster extent threshold of Family-Wise Error (FWE) corrected $P < 0.05$.

For the ROI analysis, we selected the amygdala, insula and vmPFC as ROIs. The vmPFC was defined as a 6 mm spherical ROI centered at MNI coordinates from previous studies (xyz = 4, 32, -5) (Phelps et al., 2004; Milad et al., 2007). The bilateral amygdala and insula were defined using the PickAtlas (Wake Forest University School of Medicine). The change in the functional amygdala-vmPFC and amygdala-insula coupling were examined using two-way mixed ANOVA models and subsequent simple effects analysis. Fear-acquisition associated changes within each participant were specifically examined by subtracting the individual time course correlation coefficient after acquisition from the correlation coefficient before acquisition (these data were subsequently converted to a normal distribution using Fisher's z transformation). To specifically examine differences in the fear acquisition associated changes, a two sample t-test on the change scores (Δ correlation coefficient) for the amygdala-vmPFC and amygdala-insula (RSFC: Rest2-Rest1) was used to compare the SD and control group.

Correlation analysis between RSFC and behavioral data

To investigate whether the change in amygdala-insula and amygdala-vmPFC RSFC could predict subjective fear and objective fear indices during acquisition, we conducted a correlation analysis between the RSFC change scores (Δ) of the amygdala-vmPFC and amygdala-insula and the fear indices (subjective fear and objective fear) within the experimental groups.

Results

Initial quality assessment of the data

Sixteen subjects were excluded from all subsequent statistical analysis (6 from the control group, 10 from the SD group) because they did not acquire fear conditioning as assessed by SCR. For the resting state analysis eight participants were further excluded due to high head motion ($n=3$, controls; $n=5$, SD). In addition, four subjects in the SD group were excluded from the resting state analysis due to having fallen asleep during the resting state data acquisition. Seven subjects ($n=4$, controls; $n=3$, SD) were excluded from the neural analysis due to being determined as outliers (>3 standard deviation from the group mean in change of amygdala-vmPFC and amygdala-insula RSFC). The three subjects (>3 standard deviation in change of RSFC) also did not acquire fear conditioning as assessed by SCR in the SD group. Thus a total of $n=54$ ($n=29$, controls; $n=25$, SD) subjects were included in the behavioral analysis and a total of $n=38$ subjects ($n=22$, controls; $n=16$, SD) were included in the neural analysis. After initial inspection of the data $n=5$ subjects were removed from the correlation analysis due to being determined as outliers in the control group (>3 standard deviation from the group mean in objective or subjective fear indices), leaving a total of $n=33$ subjects ($n=17$, controls; $n=16$, SD) for the correlation analysis.

Behavioral results

With respect to the subjective fear as assessed via fear ratings, two-way mixed ANOVA analysis including group (control group vs SD group) as between subject factor and the type of the CS (CSa+, CSb+ and CS-) as within subject factor revealed a significant interaction effect [$F(2, 51)=3.27, P<0.05$]. In the control group, the fear ratings were as follows: CSa+: $M=4.27, SD=1.02$, CSb+: $M=4.25, SD=1.19$, CS-: $M=1.31, SD=0.58$. In the SD group, the fear ratings were as follows: CSa+: $M=4.84, SD=0.77$, CSb+: $M=4.80, SD=0.77$, CS-: $M=1.31, SD=0.57$. Subsequent simple effect analysis, revealed that in the control group fear ratings for CSa+ [$t(28)=15.07, P<0.001$] were significantly higher than that for CS-, as well as to the CSb+ compared to CS- [$t(28)=13.18, P<0.001$]. Fear ratings did not significantly differ between CSa+ and CSb+ [$t(28)=0.09, P=0.93$]. Additionally, in the SD group, fear ratings for the CSa+ [$t(24)=19.13, P<0.001$] were significantly higher than that for the CS-, as well as for the CSb+ compared to CS- [$t(24)=24.68, P<0.001$]. Again, fear ratings did not differ for the CSa+ and the CSb+ [$t(24)=0.27, P=0.79$]. Simple effect analysis targeting between group differences revealed that the SD group reported higher subjective fear ratings than the control group for both, the CSa+ [$t(52)=2.66, P<0.01$] as well as for the CSb+ [$t(52)=2.29, P<0.05$]. Importantly, the groups reported similar fear ratings for CS- [$t(52)=0.04, P=0.97$] (Figure 2A), arguing against unspecific effects of SD on fearfulness.

With respect to the autonomic fear response as assessed by the SCR two-way mixed ANOVA analysis including group

(control group vs SD group) as between subject factor and the type of the CS (CSa+, CSb+ and CS-) as within subject factor revealed a significant interaction effect [$F(2, 51)=3.29, P<0.05$]. In the control group, the SCR was as follows: CSa+: $M=0.51, SD=0.07$, CSb+: $M=0.50, SD=0.08$, CS-: $M=0.26, SD=0.08$. In the SD group, the SCR was as follows: CSa+: $M=0.68, SD=0.22$, CSb+: $M=0.64, SD=0.24$, CS-: $M=0.34, SD=0.21$. Subsequent simple effect analysis revealed that in the control group the SCR in response to the CSa+ [$t(28)=13.16, P<0.001$] were significantly higher than that for the CS-, as well as in response to the CSb+ compared to the CS- [$t(28)=16.01, P<0.001$]. Fear ratings did not significantly differ between CSa+ and CSb+ [$t(28)=0.61, P=0.72$]. Similarly, in the SD group, SCR for CSa+ [$t(24)=8.65, P<0.001$] was significantly higher than that for CS-, as well as to CSb+ compared to CS- [$t(24)=12.67, P<0.001$]. Again, fear indices were not significantly different for the CSa+ and the CSb+ [$t(24)=1.07, P=0.17$]. Examination of between-group differences using simple effect analysis demonstrated higher autonomic fear responses in the SD group compared to the control group for both the CSa+ [$t(52)=3.34, P<0.005$], as well as for the CSb+ [$t(52)=2.64, P<0.01$]. Importantly, the groups displayed equivalent fear autonomic responses towards the CS- [$t(52)=1.57, P=0.12$], arguing against unspecific effects of SD on autonomic fear responses. Together, the behavioral indices indicate that both groups successfully acquired the fear response, but that both, subjective and objective fear indices during acquisition were enhanced following SD (Figure 2B).

Resting-state fMRI results

Changes in RSFC before and after fear acquisition. Next we examined how SD affects the fear acquisition induced changes in the amygdala RSFC networks during consolidation. Whole-brain voxel-wise analysis by means of a two-way mixed ANOVA models including group (SD group vs control group) as between subject factor and the REST acquisition time point (Rest1 vs Rest2) as within subject factor revealed a significant interaction effect [$F(1, 72)=7.78, P=0.01, FWE$ corrected] located in the insula and vmPFC. These regions have been previously found to be engaged in fear memory consolidation (Feng et al., 2013; Feng et al., 2014). Subsequent simple effect analysis revealed that the functional connectivity between the amygdala-insula was increased in the SD group [Left insula: peak voxel coordinates, $(-30, 30, 3), t(15)=7.31$, cluster FWE corrected $P<0.05, k=67$; Right insula: peak voxel coordinates, $(51, 15, 3), t(15)=5.95$, cluster FWE corrected, $P<0.001, k=133$] (Figure 3A), whereas the RSFC between amygdala and vmPFC was increased in the control group [peak voxel coordinate, $(-9, 54, 6), t(21)=4.77$, cluster FWE corrected, $P=0.001, k=144$] (Figure 3B).

Given the importance of the amygdala-vmPFC and the amygdala-insula pathways during fear acquisition and consolidation (Cisler et al., 2014; Feng et al., 2014; Nicholson et al., 2016; Van Marle et al., 2010), effects of SD on these pathways were further explored using a ROI-to-ROI approach comparing the time course correlation coefficient between amygdala and vmPFC, amygdala-insula respectively. For this analysis, ROIs were selected on the basis of prior research (vmPFC, 4, 32, -5) with the radius of 6 mm (Agren et al., 2012; Milad et al., 2007; Phelps et al., 2004) and anatomical bilateral amygdala and insula from the Pickatlas (Wake Forest University School of Medicine). Two-way mixed ANOVA with the factors group (control group vs SD group) and the RSFC pathway (amygdala-vmPFC vs amygdala-insula) revealed a significant group \times RSFC interaction effects [$F(1, 35)=17.21, P<0.001$]. In line with the voxel-wise approach,

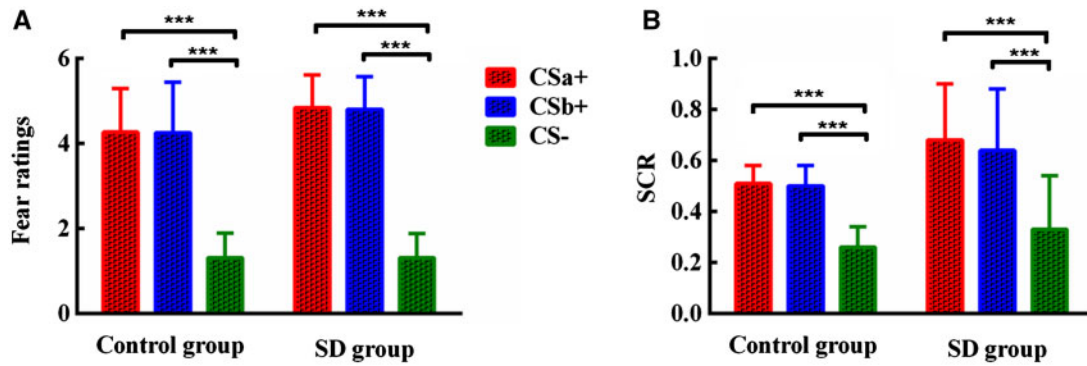


Fig. 2. (A, B) There was much more fear ratings and SCR in responses to CSa+ compared to CS-, as well as to CSb+ compared to CS- in both group, but the level of fear ratings and SCR to CSa+ and CSb+ was equivalent. Moreover, in the SD group, participants showed stronger fear response than that in the control group for CSa+, as well as for CSb+, but there was no significant difference in responses to CS- between control group and SD group.

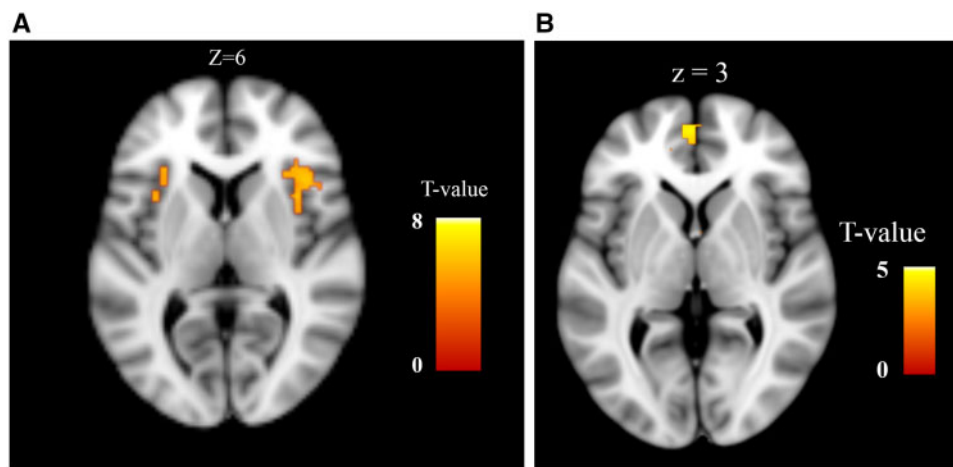


Fig. 3. (A) For voxel wise analysis, there was stronger RSFC between amygdala and insula for Rest2 (in fear consolidation window) than that for Rest1(baseline) in the SD group. (B) However, there was stronger RSFC between amygdala and vmPFC for Rest2 than that for Rest1 in the control group.

a simple effect analysis revealed that fear acquisition induced functional connectivity changes between the amygdala and insula were greater in the SD relative to the control group ($P < 0.001$), whereas changes in amygdala-vmPFC functional connectivity were greater in the control group relative to the SD group ($P < 0.05$) (Figure 4). Additionally, to verify whether the change RSFC difference between two groups were due to SD, we performed two sample t-test separately comparing the pre-acquisition (Rest1) and the post-acquisition (Rest2) data between the experimental groups. Importantly, this analysis revealed that there were no differences of amygdala-insula ($P = 0.57$) and amygdala-vmPFC ($P = 0.11$) RSFC between the groups at Rest1, arguing against unspecific effects of SD on baseline neural activity. However, the amygdala-insula showed greater RSFC in the SD group than that in the control group at Rest2 ($P < 0.05$), while the amygdala-vmPFC showed greater RSFC in the control group than that in the SD group at Rest2 ($P < 0.05$). The results suggest that SD was associated with increased RSFC of the amygdala-insula and interfered with amygdala-vmPFC connectivity during fear consolidation.

Together, the neural results indicate that the interaction of the amygdala with the insula and vmPFC during fear consolidation is susceptible to SD, possibly reflecting enhanced impact of fear acquisition on amygdala-insula salience pathways and concomitantly inhibited vmPFC top-down control of the amygdala during fear memory consolidation.

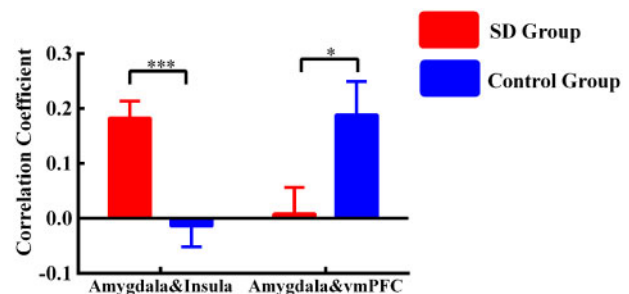


Fig. 4. The difference change RSFC of vmPFC-amygdala and amygdala-insula between Rest1 and Rest2 for control group and for SD group.

The change in RSFC predicts subjective and objective fear. To further examine whether SD affects the associations between subjective and objective fear indices during the acquisition stage and subsequent fear consolidation in the amygdala, vmPFC and insula pathways, group-specific correlations between the behavioral and neural indices were examined. The correlation analysis demonstrated that changes in amygdala-vmPFC functional connectivity were negatively correlated with subjective (fear ratings, $r = -0.47$, $P < 0.05$) and objective (SCR, $r = -0.56$, $P < 0.05$) fear indices in the control group (Figure 5A and B), Whereas changes in both behavioral indices (fear ratings, $r = 0.67$, $P < 0.005$; SCR,

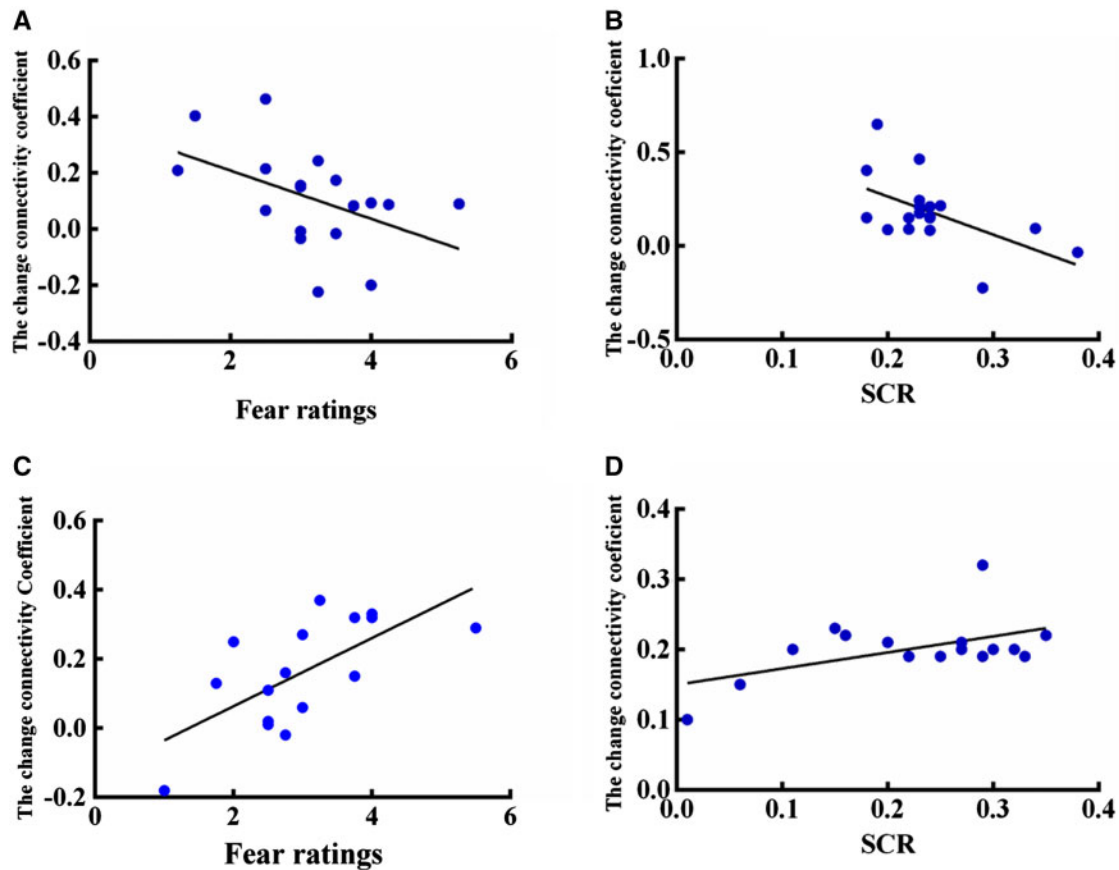


Fig. 5. (A, B) The correlation analysis showed that the change RSFC between vmPFC and amygdala was negatively correlated with the subjective fear ratings and SCR in control group. (C, D) However, the change RSFC between amygdala and insula was positively correlated with the subjective fear ratings and SCR in SD group.

$r=0.53$, $P<0.05$) were positively correlated with amygdala-insula functional connectivity in the SD group (Figure 5C and D). The finding suggested that SD may lead to over consolidation of fear memory (increased amygdala-insula RSFC) and weaken the top-down ability of vmPFC to regulate the amygdala during the fear memory consolidation window.

Effects of SD at the extinction stage

An additional exploratory analysis examined the effects of SD during fear acquisition on extinction on day 3. A two-way mixed ANOVA on the subjective fear indices (ratings) that included group (control group vs SD group) as between subject factor and type of the CS (CSb+ and CS-) as within subject factor revealed a significant interaction effect between two factors [$F(1, 52)=3.91$, $P<0.05$]. Specifically, the following results were obtained: in the control group, the subjective fear ratings of CSb+ and CS- were as follows: CSb+, $M=1.58$, $SD=0.61$; CS-, $M=1.32$, $SD=0.72$. In the SD group, the subjective fear ratings were as follows: the CSb+, $M=2.15$, $SD=1.21$; CS-, $M=1.37$, $SD=0.58$. Subsequent simple effect analysis showed that participants in the SD group had greater fear ratings than subjects in the control for the CSb+ [$t(52)=2.13$, $P<0.05$]. However, there was no significant between-group difference with respect to the CS- [$t(52)=0.25$, $P=0.8$]. A further simple effect analysis that examined within-group differences between the CSb+ and the CS- revealed that controls did not report differential fear ratings for the CSb+ and the CS- [$t(28)=1.70$, $P=0.18$], whereas the

SD group reported a significantly higher fear rating for the CSb+ as compared to the CS- [$t(24)=3.64$, $P<0.001$].

A two-way mixed ANOVA analysis on the objective fear indices (SCR) at the stage of extinction that included group (control group vs SD group) as between subject factor and CS-type (CSb+ and CS-) as within subject factor revealed a significant interaction effect between the factors [$F(1, 52)=3.34$, $P<0.05$]. Specifically, controls exhibited CSb+, $M=0.16$, $SD=0.12$; CS-, $M=0.15$, $SD=0.15$; whereas the SD group exhibited CSb+, $M=0.27$, $SD=0.25$; CS-, $M=0.22$, $SD=0.18$. Subsequent simple effect analysis demonstrated that participants in the SD group exhibited a greater SCR than controls for the CSb+ [$t(52)=2.01$, $P<0.05$], in the context of no between-group differences for the CS- [$t(52)=1.26$, $P=0.22$]. An additional simple effect analysis on the within-group differences for the stimuli revealed that controls did not show significantly different SCRs for the CSb+ as compared to the CS- [$t(28)=0.20$, $P=0.89$], whereas the CSb+ elicited a significantly stronger SCR relative to the CS- in the SD group [$t(24)=2.14$, $P<0.01$]. Together, the findings from this exploratory analysis indicate that the effects of SD on the acquisition of fear were mirrored during extinction following normal sleep.

Discussion

To examine effects of SD on the intrinsic RSFC networks of the amygdala during fear consolidation, we employed a RSFC approach assessing intrinsic brain activity before and immediately following fear acquisition under conditions of 24 h of SD,

and additionally examined whether the fear acquisition associated RSFC changes in the fear consolidation window can be predicted by subjective and objective fear indices during the stage of fear acquisition. On the behavioral level, participants in SD group demonstrated increased fear ratings and autonomic fear reactivity at the stage of fear acquisition relative to the control group. Analysis of amygdala RSFC networks revealed that the SD group exhibited enhanced fear acquisition associated amygdala-insula connectivity relative to the control group. In accordance with this pattern, subjective and objective indices of fear during the acquisition stage were positively correlated with fear acquisition associated changes in amygdala-insula connectivity in the SD group.

Consistent with our hypotheses, SD was associated with enhanced fear experience and autonomic fear reactivity during the stage of fear acquisition. Importantly, the specificity of the effect was confirmed by a lack of sleep associated changes of fear reactivity to the CS-. The present findings of enhanced fear acquisition following SD converge with previous findings reporting an SD-associated generalized failure to habituate during fear acquisition (Peters et al., 2014), lack of decrease in negative emotional reactivity (van der Helm et al., 2011), and enhanced impulsivity (Anderson and Platten, 2011). Together with the present findings, this suggests that an SD-induced increase in the reactivity to negative emotional stimuli might enhance fear acquisition. Further, we also found that SD also enhanced subjective and objective indices at fear extinction stage following 24h sleep recovery. A study found that sleep deprivation selectively impaired the accurate judgment of human facial emotions, especially threat relevant (anger) categories (Van Der Helm et al., 2010). Another study found that SD impaired the ability of discrimination to threat face stimuli. Specifically, SD participants significantly categorized more faces as threatening and fewer faces as nonthreatening relative to the sleep participants (Goldstein-Piekarski et al., 2015). The present findings indicated that SD impaired the extinction effect at fear extinction stage following 24h sleep recovery.

More importantly, enhanced fear acquisition following SD was accompanied by a lack of enhanced fear-acquisition associated amygdala-vmPFC connectivity (as observed in the control group) and increased amygdala-insula connectivity. The amygdala plays a key role in threat reactivity and fear learning, whereas converging evidence from imaging and lesion studies suggest a critical role of the vmPFC in the top-down regulation of amygdala reactivity (Amano et al., 2010; Knapska et al., 2012; Motzkin et al., 2015), thus promoting emotion regulation and behavioral control (Sokol-Hessner et al., 2012; Wagner and Heatherton, 2013). In line with previous research (Feng et al., 2014), a negative association between fear indices during acquisition and changes in this pathway during consolidation was observed in the controls, suggesting a particular involvement of the amygdala-vmPFC pathway in fear consolidation. In line with the present observation of a disruption of enhanced amygdala-vmPFC connectivity following SD, a wealth of previous studies demonstrated that SD was associated with reduced prefrontal-amygdala functional connectivity putatively reflecting an SD-induced disruption of top-down control of emotion. The specific vulnerability of the vmPFC and its functional relevance to SD-associated impairments is emphasized by studies reporting pronounced SD-associated decreases in vmPFC cerebral energy metabolism (Thomas et al., 2000), decreased functional connectivity between vmPFC and amygdala in response to negative aversive stimuli (Yoo et al., 2007), as well as associations between SD-induced mood changes and

intrinsic amygdala-mPFC connectivity (Lei et al., 2015). Furthermore, deficient vmPFC-mediated inhibition of amygdala reactivity is considered a key pathological feature in disorders characterized by exaggerated negative affect and deficient emotion regulation, particularly mood and anxiety disorders (Cha et al., 2014; Milad et al., 2006; Quirk and Gehlert, 2003; Rauch et al., 2006).

Whereas SD inhibited vmPFC's modulation of the amygdala, it increased fear-acquisition associated connectivity changes in amygdala-insula coupling. Together with the dorsal ACC, the insula and amygdala are at the core of the affective salience networks. The amygdala and insula co-activate during arousal processing, including early subconscious arousal reactivity (Brooks et al., 2012). Moreover, the intrinsic connectivity between these regions has been associated with integrating interoceptive signaling with emotional awareness (Simmons et al., 2013). Connectivity between the amygdala and insula furthermore has been repeatedly associated with individual differences in anxiety, including state and trait anxiety (Baur et al., 2013), childhood anxiety (Qin et al., 2014), as well as symptom severity in patients with generalized anxiety disorder (Roy et al., 2013). Accumulating evidence suggests that SD increases the noradrenergic tone promoting an exaggerated and over-generalized hyper-reactivity in the core nodes of the affective salience and arousal networks, including the amygdala and insula (Franzen et al., 2009; Goldstein-Piekarski et al., 2015; Goldstein et al., 2013; Goldstein and Walker, 2014). Together with the previous findings, the current results therefore suggest that SD-induced hyper-connectivity in the amygdala-insula pathway reflects a failure to efficiently regulate arousal and interoceptive signaling during fear consolidation. The observed association between higher fear indices during the acquisition stage and enhanced connectivity in the amygdala-insula pathway following SD might reflect increased bottom-up arousal and salience signaling of the amygdala during fear consolidation.

In the context of the important role of sleep disturbances in PTSD it seems noteworthy to mention that exaggerated arousal and biased salience processing have been proposed a key factor for the maintenance of PTSD (Buckley et al., 2000; Shin and Liberzon, 2010; Sripada et al., 2012) and exaggerated amygdala-insula connectivity has repeatedly been observed in patients with PTSD (Fonzo et al., 2010; Nicholson et al., 2016; Rauch et al., 2000; Shin et al., 2005).

The present findings need to be considered in the context of some limitations, particularly the fact that no objective methods (ambulatory electroencephalographic or actigraphic monitoring) were applied to monitor sleep and sleep quality before and during the experiment. Moreover, SD might be associated with unspecific increases in stress and future studies should consider to acquire salivary cortisol to further disentangle effects of enhanced stress from effects specifically related to SD. This seems to be particularly relevant in the light of a previous study reporting increased amygdala-dACC and amygdala-insula RSFC following experimentally-induced moderate psychological stress (Van Marle et al., 2010). Furthermore, future studies should consider to include different SD intervals to determine whether effects on fear processing change during the course of SD and should consider to re-examine amygdala-connectivity in SD subjects following a night of regular sleep to evaluate the reversibility of the effects. Finally, the SD was associated with increased negative and decreased positive emotions as assessed by the PANAS. Therefore, we cannot rule out that differences in mood might have contributed to the observed

differences in fear acquisition. Importantly, there were no differences in the RSFC before the conditioning procedure (Rest 1) arguing against strong confounding effects of mood differences on the neural indices.

Summarizing, the present study investigated the effects of SD on fear acquisition and associated changes in amygdala RSFC at the stage of fear memory consolidation and found enhanced fear indices during acquisition that were accompanied by a disruption of amygdala coupling with the vmPFC and enhanced amygdala-insula coupling during fear consolidation. In the light of the proposed role of sleep disturbances in the development and maintenance of PTSD, the findings may help to understand the neural basis of the detrimental effects of sleep disturbances in the disorder.

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Conflict of interest. None declared.

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