One night of partial sleep deprivation affects habituation of hypothalamus and skin conductance responses

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Fear conditioning and subsequent extinction are the dominant experimental models for the etiology and maintenance of normal and pathological anxiety (Pape and Pare 2010; Rauch et al. 2006). Accordingly, a considerable number of studies have documented deficient extinction processes in patients with post-traumatic stress disorder (Blechert et al. 2007; Milad et al. 2009; Wessa and Flor 2007), panic disorder (Michael et al. 2007), and other anxiety disorders, including specific phobias (Lissek et al. 2005). Studies on the underlying neural circuitry identify the amygdala as essential for the acquisition and expression of conditioned fear (Phelps and LeDoux 2005), as well as for extinction learning (Phelps et al. 2004). Recall and expression of the extinction memory seem to involve the ventromedial prefrontal cortex (vmPFC) (Kalisch et al. 2006; Milad et al. 2007; Phelps et al. 2004).

A combination of these two lines of research on fear learning and sleep disturbances in an experimental context could help clarify whether sleep plays a causal role in the development and eventually maintenance of anxiety (Germain et al. 2008; Levin and Nielsen 2007). Initial evidence for an interaction between sleep disturbances and fear learning comes from the animal literature and suggests a bidirectional relationship: studies in rodents have shown disrupted sleep after fear conditioning (DaSilva et al. 2011; Jha et al. 2005; Kumar and Jha 2012; Sanford et al. 2003), whereas successful extinction learning restored sleep to normal levels (Wellman et al. 2008). Critically, overnight changes in fear memory were correlated specifically with theta coherence in limbic and medial prefrontal regions during rapid eye movement (REM) sleep (Popa et al. 2010). Moreover, it has been shown that pretraining REM SD has detrimental effects on avoidance learning in rats (Gruart-Masso et al. 1995), further suggesting that REM sleep could be of particular interest in this context. Although analogous research in humans is only just starting, the clinical data mentioned above indicate that sleep disturbances can predate and predict the subsequent onset of clinical anxiety.

Therefore, the goal of the present study was to investigate whether one night of partial SD, depriving participants of the second half of the night, rich in REM sleep, would impact subsequent fear-learning processes. First, partial SD may alter nonassociative learning processes at the physiological level during fear conditioning. For example, a daytime nap session was found to promote physiological intersession habituation to repeatedly presented, aversive images, an effect that was reduced by the occurrence of REM sleep (Pace-Schott et al. 2011). In a different study, subjects who showed diminished habituation in their skin conductance response (SCR) to elec-

INCREASING EVIDENCE ATTESTS to the prevalence of highly debilitating sleep disturbances in anxiety and mood disorders (Ohayon 2003; Ohayon and Roth 2003; Ohayon and Shapiro 2000). Yet, the etiological role of sleep in the development of anxiety remains unclear. Epidemiological data with retrospective measurements indicate that sleep disturbances often occur concurrently or after the development of an anxiety disorder (Ohayon and Roth 2003). However, self-reported sleep disturbances in the 2 wk before a traumatic event predict an increase in affective and anxiety symptomatology 3 mo later (Bryant et al. 2010). In healthy subjects, acute sleep deprivation (SD) has been shown to increase subjective anxiety (Babson et al. 2010). More specifically, SD is associated with self-reported physiological symptoms of anxiety (Kahn-Greene et al. 2007) and was reported to lower the subjective threshold for stress perception (Minkel et al. 2012).

Address for reprint requests and other correspondence: V. I. Spoormaker, Neuroimaging Research Group, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany (e-mail: spoormaker@mpipsykl.mpg.de).

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Peters AC, Blechert J, Sämann PG, Eidner I, Czisch M, Spoormaker VI. One night of partial sleep deprivation affects habituation of hypothalamus and skin conductance responses. J Neurophysiol 112: 1267–1276, 2014. First published June 11, 2014; doi:10.1152/jn.00657.2013.—Sleep disturbances are prevalent in clinical anxiety, but it remains unclear whether they are cause and/or consequence of this condition. Fear conditioning constitutes a valid laboratory model for the acquisition of normal and pathological anxiety. To explore the relationship between disturbed sleep and anxiety in more detail, the present study evaluated the effect of partial sleep deprivation (SD) on fear conditioning in healthy individuals. The neural correlates of 1) nonassociative learning and physiological processing and 2) associative learning (differential fear conditioning) were addressed. Measurements entailed simultaneous functional MRI, EEG, skin conductance response (SCR), and pulse recordings. Regarding nonassociative learning, partial SD resulted in a generalized failure to habituate during fear conditioning, as evidenced by reduced habituation of SCR and hypothalamus responses to all stimuli. Furthermore, SCR and hypothalamus activity were correlated, supporting their functional relationship. Regarding associative learning, effects of partial SD on the acquisition of conditioned fear were weaker and did not reach statistical significance. The hypothalamus plays an integral role in the regulation of sleep and autonomic arousal. Thus sleep disturbances may play a causal role in the development of normal and possibly pathological fear by increasing the susceptibility of the sympathetic nervous system to stressful experiences. Sleep deprivation; fear conditioning; skin conductance; hypothalamus; fMRI
trical shocks during fear conditioning did not achieve REM sleep during a subsequent nap opportunity (Spoormaker et al. 2010). Given that the hypothalamus plays a critical role in sleep regulation, it is likely that the sudomotor fibers that project from the hypothalamus to the ventrolateral spinal sympathetic tract (Boucsein 2012) mediate these effects. If SD acts on such basic physiological levels, effects of SD should be generalized, i.e., affect all stimulus types [conditioned (CS⁺), safety (CS⁻), and unconditioned (US)].

Second, partial SD could impact associative learning processes supported by the amygdala. It has been reported that compared with a waking day, a night of sleep resulted in decreased behavioral and amygdala, responding to emotional stimuli seen previously (van der Helm et al. 2011). This decrease was significantly correlated with the reduction in REM sleep gamma EEG activity. Another study found that one night of total SD was associated with enhanced amygdala activation to negative stimuli and a significant decrease of functional connectivity between amygdala and the mPFC (Yoo et al. 2007), a region with extensive inhibitory projections to the amygdala (Walker 2009). If SD impacted associative learning in this way, it should have a differential effect on CS⁺ and CS⁻.

In short, we studied the interaction of sleep and fear conditioning on two response levels. If SD affected nonassociative learning processes, then we would expect hypothalamus and brain stem activity, as well as physiological responses to all stimuli, to be different. However, if SD affected associative learning processes, then we would expect amygdala activity and SCR to be differentially affected across stimuli, resulting in either enhanced or reduced differential conditioning.

METHODS AND MATERIALS

Participants

Thirty-two healthy volunteers (17 women, aged 21–30 yr, mean age 24.19 ± 2.89 yr) took part in the study. They were recruited from the local universities in Munich and passed a telephone screening before participation. The sample consisted of students and three trainees with normal or corrected-to-normal vision. German-native speakers without any history of neurological or psychiatric diseases and sleep disorders were included. All participants were required to be nonsmokers and right handed, as assessed by the Edinburgh Handedness Inventory (Oldfield 1971). Standard MRI exclusion criteria, such as metal implants and claustrophobia, were applied. Students of medicine and psychology were precluded from participation because of possible insights into the experimental paradigm.

After having been explained the study procedure, subjects provided their written, informed consent and were financially reimbursed. The experimental protocol conformed to the Declaration of Helsinki and was approved by a local Ethical Review Committee. Four participants (two men, two women) had to be excluded, one due to a history of mild depression, including psychological treatment, and three for technical reasons.

Behavioral Paradigm

The experimental task was performed in the magnetic resonance scanner and involved a discriminatory fear-conditioning paradigm with a partial reinforcement schedule, adapted from Delgado et al. (2008). It comprised three sections: first, subjects underwent a short habituation section, in which a yellow and a blue square were presented twice each for 8 s with intertrial intervals (ITIs) of 5–8 s. Second, participants were conditioned to a specific square color by means of a mild electric shock, which followed stimulus onset with a delay of 7.8 s in 33% of trials. This square subsequently served as the CS⁺, whereas the other one signaled CS⁻. Participants were told that squares might be accompanied by shocks but were unaware of the specific color contingency. Both types of squares were displayed 12 times in pseudorandom order (less than or equal to three repetitions of the same stimulus) for 8 s, with an ITI jittered between 8 s and 12 s. The third section was designed to assess the impact of partial SD on the cognitive regulation of acquired fear (data not shown). The entire task lasted ~20 min.

Procedure

The study protocol followed a between-subject design with one night of partial SD as the experimental manipulation. Participants completed a screening session and a testing session. Twenty-four hours before the testing session, they were required to refrain from consuming alcohol and caffeine to prevent masking of possible SD effects.

In the screening session, subjects underwent an anatomical scan to exclude neurological disorders and were randomized into the control group (normal sleep) or the experimental group (instructed to wake up 4 h before their usual rising time). The screening and testing sessions were scheduled five nights apart. During that time, all participants had to maintain a regular sleep schedule, as documented by sleep protocols and actigraphy. The testing session started at 9 AM with a short reminder of the task instructions. For recordings simultaneous to functional scans, two SCR electrodes were attached to the phalanges of the subjects’ left index and middle finger and an EEG cap placed on their scalp. Two gold electrodes were applied to their right wrist for shock administration. Heart rate was measured by pulse oximetry on the left thumb. The session concluded with the completion of questionnaires, as well as estimates of the perceived shock probability and valence ratings for each square. Refer to Fig. 1 for an overview of the experimental procedure.

Electrical Stimulation

For the administration of electric shocks, a Digitimer stimulator (model DS7; Digitimer, Hertfordshire, UK) was used. Via custom-made gold electrodes suited for stimulation in the magnetic resonance environment, a pulse of 2 ms, with intensities ranging from 4 to 15 mA, was applied to the back of the right hand [mean (M) = 7.36, standard deviation (std. dev.) = 2.93]. As the threshold for pain varies interindividually, a staircase protocol was used to set the shock intensity at a level that was uncomfortable but not painful. After the testing session, participants provided a subjective rating of shock aversiveness on a visual analog scale.

Questionnaires

A comprehensive questionnaire battery was applied to test for comparability of groups and to address potential confounds. Personality measures included the Big Five Inventory (Lang et al. 2001), the Tridimensional Personality Questionnaire (Weyers et al. 1995), and the State and Trait Anxiety Inventory (Laux et al. 1981). To evaluate sleep quality and chronotype, the Pittsburgh Sleep Quality Index (PSQI) (Riemann and Backhaus 1996) and the Morningness-Eveningness Questionnaire (D-MEQ) (Griefahn et al. 2001) were administered. Other questionnaires comprised the Beck Depression Inventory (Schmitt et al. 2003), the Emotion Regulation Questionnaire (Abler and Kessler 2009), and the Social Desirability Scale-17 (Stöber 1999).

Behavioral Ratings

To evaluate whether participants learned the CS-US contingency, two-way [group repeated over stimulus (CS⁺, CS⁻)] ANOVAs were
performed on 1) estimates of shock probability and 2) CS-valence ratings. In case of nonsphericity, the Greenhouse-Geisser correction was applied.

Sleep Measures and Analysis

Actigraphy was measured on the five nights between the screening and the testing session by an ActiSleep monitor (ActiGraph, Pensacola, FL). Data were extracted and analyzed by the software package ActiLife v5.10.0 (ActiGraph), resulting in the variables bed time, rising time, time in bed, and total sleep time (TST) for the four control nights and for the experimental night. Between-group differences were tested with independent sample t-tests (equal variances assumed unless mentioned otherwise). Furthermore, activity levels were quantified based on counts per minute (CPM) on the vertical axis and categorized according to Freedson et al. (1998). This resulted in the following activity level categories: "Sedentary" (0–99 CPM), "Light" (100–759 CPM), "Lifestyle" (760–1,951 CPM), "Moderate" (1,952–5,724 CPM), "Vigorous" (5,725–9,498 CPM), and "Very Vigorous" (from 9,499 CPM) (Freedson et al. 1998). These were tested for between-group differences in the experimental night by Mann-Whitney U-tests.

To investigate whether there was a shift in circadian rhythm dependent on group, a two-way [group repeated over sleep measure (PSQI, actigraphy, sleep diaries)] ANOVA was performed on the pre-experimental nights. This analysis was repeated for the experimental night for the variable bed time only.

SCR Recording and Analysis

SCR was sampled at 5 kHz by means of a BrainAmp ExG MR amplifier (Brain Products, Gilching, Germany), before being down sampled to 50 Hz, z transformed, and baseline corrected. The SCR was defined as the peak-to-trough difference in SCR of the maximal positive deflection in three time windows after stimulus onset: 0.5–4.5 s [first interval response (FIR)], 4.5–8.5 s [second interval response (SIR)], and 8.5–12.5 s [third interval response (TIR)] (Boucsein 1992). Due to skewness, individual raw SCR values were square-root transformed. For all task sections, the CS− and CS+ presentations were averaged over three consecutive trials. TIR segments, containing shocks, allowed us to assess the habituation of the unconditioned response (UR) by comparing two shocks in the first half of acquisition with two shocks in the second half of acquisition. Because a differential, anticipatory SCR (FIR) is typically regarded as proof for differential conditioning in the literature, a three-way (group repeated over stimulus and time) ANOVA was performed on the FIR. Next, a four-way (group repeated over stimulus × response window × time) ANOVA evaluated whether fear conditioning differed between groups as a function of response window (FIR, SIR, TIR). Because CS+ trials contained less TIR (due to UR in this window) than CS− trials, we included the first vs. last three trials (FIR1,4, SIR1,4, TIR1,4) in this ANOVA. For the examination of correlations between SCR and other data modalities, SCR data were reduced by principal component analysis (PCA; with varimax rotation).

Pulse Recording and Analysis

Pulse data were collected at 100 Hz and z transformed. Assessment applied to the number of beats during 7.8 s of CS presentation (thus excluding shock applications). Analogous to SCR analyses, 12 CS− and 12 CS+ presentations were averaged over three consecutive trials to receive four values that tracked changes in pulse behavior over time in the conditioning section. A three-way (group repeated over stimulus and time) ANOVA was performed to evaluate whether the heart rate increased in response to the CS+ relative to the CS− with time and whether this increase differed between groups.

EEG Acquisition and Analysis

Since the experimental procedure may affect vigilance levels, we used simultaneous EEG during the functional MRI (fMRI) runs as a control measure. The set-up comprised a custom-made, 12-channel EEG cap that conformed to the international 10/20 system and included reference and ground electrodes (EasyCap, Herrsching, Germany). With the use of BrainVision Analyzer 2.0 software (Brain Products), the EEG signal was postprocessed to eliminate artifacts through mean artifact-template subtraction, Independent Component Analysis, filtering (0.5–30 Hz), and semiautomatic bad-segment rejection. Fast Fourier transforms for each 2.5-s segment were performed, and alpha (8–12 Hz) to full (2–12 Hz) band ratios for the F3, F4, O1, and O2 channels were computed for each volume. These ratios were used for vigilance scoring, according to Olbrich et al. (2009). For each task section, ratios were summarized into 10 equally spaced time intervals and entered into a three-way (group repeated over time and channel) ANOVA.

fMRI Acquisition and Analysis

Whole-brain MRI data were obtained on a 3-Tesla scanner (Discovery MR750; General Electric, Waukesha, WI) with a 12-channel head coil. Functional images were collected with an echo-planar imaging (EPI) pulse sequence [30 ms echo time, 2,500 ms repetition time, interleaved slice order, 90° flip angle, 24 cm field of view, 96 × 96 matrix with parallel imaging (Array Spatial Sensitivity Encoding Technique) with a factor of two, reconstructed images interpolated to 128 × 128 matrix]. Each volume consisted of 42 anterior–posterior-
oriented slices of 2.5 mm thickness and a final in-plane resolution of 2 × 2 mm², with a slice gap of 0.5 mm.

Functional data were preprocessed and statistically analyzed with SPM8 [www.fil.ion.ucl.ac.uk/spm; Statistical Parametric Mapping (SPM)]. The first four volumes were discarded to account for T1 saturation. The remaining scans were slice-time corrected and realigned to the volume mean using rigid body transformation. Images were normalized to the EPI template of the Montreal Neurological Institute, resampled to a voxel resolution of 2 × 2 × 2 mm³, and spatially smoothed with a three-dimensional isotropic Gaussian kernel (full width at half maximum: 5 mm). Functional data were high-pass filtered with a cutoff period of 300 s.

On the single-subject level, a general linear convolution model for blood oxygen level-dependent (BOLD) signal changes was applied to the time course of each voxel. Nine nuisance variables were entered as parameters of no interest: six affine motion-correction regressors computed during realignment and the average signal fluctuations of three predefined regions extracted with SPM toolbox MarsBaR 0.41. These comprised a cerebrospinal fluid (CSF) mask, a deep white matter sphere, and a deep CSF sphere, both with 5 mm radius. Three different CS events and their time modulation were defined as regressors of interest: CS⁺ with shock, CS⁻ without shock, and CS⁻.

On the group level, random effects analyses were performed. For each subject and stimulus regressor, statistical parametric maps of a simple +1 contrast were computed and entered into full-factorial and flexible-factorial ANOVAs. Analyses comprised a two-way (group repeated over stimulus) ANOVA of the three stimulus regressors and an equivalent ANOVA on the time modulation/stimulus regressor. A linear time modulation was chosen over a split-half comparison because of the loss of power inherent to such an approach and because of the resulting issues, with a limited number of regressors for the flexible factorial ANOVA, as implemented in SPM. Contrast estimate values were extracted from a 5-mm sphere around the peak voxel in SPM8 using the eigenvariate function. The reported statistical maps were sampled at a threshold of P < 0.001; reported clusters had a whole-brain corrected significance with cluster P values < 0.05 after correction for familywise error under consideration of nonstationary smoothness.

RESULTS

Sample Characteristics and Manipulation Check

There were no significant differences between the SD group and the control group regarding age [t(26) = 0.08, P > 0.10] or gender [χ²(1) = 0.14, P > 0.10]. As shown in Table 1, both groups had equivalent scores on measures of personality, anxiety, depression, sleep quality, emotion regulation, and social desirability. SD and control participants only differed in their chronotype, with the former tending toward a moderate morningness type and the latter toward a moderate eveningness type [t(26) = 2.06, P = 0.050]. Therefore, all analyses were repeated with the D-MEQ chronotype scores as a covariate.

We evaluated further whether SD affected shock-intensity levels but did not observe any significant group differences for objective shock intensity [in mA; mean of the SD group (Mₕₔ) = 7.00 (std. dev. = 2.95); mean of the control group (Mₐₖ) = 7.34 (std. dev. = 2.83); t(29) = −0.33, P = 0.743] or subjective shock ratings [Mₕₔ = 52.60 (std. dev. = 17.21); Mₐₖ = 47.31 (std. dev. = 19.67); t(29) = 0.79, P = 0.434].

Both actigraphy and sleep diaries verified that TST averaged across the four nights leading up to the second measurement did not differ significantly between groups [actigraphy: t(26) = 0.55, sleep diaries: t(26) = 1.06, P > 0.10]. In contrast, in the experimental night, participants in the SD group slept significantly less than those in the control group [actigraphy: t(26) = 6.05, P < 0.001; sleep diaries: t(26) = 12.98, P < 0.001]. Accordingly, in the experimental night, the SD group had an earlier rising time compared with the control group [actigraphy: t(26) = 6.19, P < 0.001; sleep diaries: t(26) = 7.76, P < 0.001], whereas the bed time was comparable [actigraphy: t(26) = −0.33, sleep diaries: t(26) = −0.76; P > 0.10; see Table 2].

Regarding motor activity during the deprivation period in the experimental night, activity levels were higher in the SD group, showing less percentage time spent in the Sedentary activity level category and more in Light, Lifestyle, and Moderate (all P < 0.005). However, SD did not result in an increase of the highest activity levels, Vigorous and Very Vigorous (both P > 0.80). There were no between-group differences in the nights preceding the experimental night (all P > 0.50).

Next, we examined whether the sleep-wake rhythms differed across groups, according to sleep measure (PSQI, actigraphy, sleep diaries): a two-way (group repeated over sleep measure) ANOVA on the recorded nights preceding the experimental night showed a significant main effect of sleep measure for bed time [F(2,48) = 8.28, P < 0.001], rising time (F = 5.53, P < 0.01), and TST (F = 7.79, P < 0.005). Neither the main effect of group nor the group × sleep measure interaction was significant for any of these dependent variables (all F ≤ 2.0, all P > 0.10). The same analysis was repeated for the experimental night for bed time only. Again, the main effect of sleep measure was significant [F(2,42) = 27.52, P < 0.001], whereas

<table>
<thead>
<tr>
<th>Table 1. Means (±SD) of background variables/group</th>
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<tr>
<td>Control Group</td>
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<td>Age</td>
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<tr>
<td>Extraversion</td>
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<td>Agreeableness</td>
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<td>Conscientiousness</td>
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<td>Neuroticism</td>
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<td>Openness</td>
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<td>TPO</td>
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<td>Novelty seeking</td>
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<td>Reward dependence</td>
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<td>STAI</td>
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<td>State anxiety</td>
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<td>Depression</td>
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<td>Sleep quality</td>
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<td>Reappraisal</td>
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<td>Suppression</td>
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<td>SD-17</td>
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<td>Social desirability</td>
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<td>D-MEQ</td>
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<td>Chronotype</td>
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SD, sleep deprivation; BFI, Big Five Inventory; TPO, Tridimensional Personality Questionnaire; STAI, State and Trait Anxiety Inventory; BDI, Beck Depression Inventory; PSQI, Pittsburgh Sleep Quality Index; ERQ, Emotion Regulation Questionnaire; SD-17, Social Desirability Scale-17; D-MEQ, Morningness-Eveningness Questionnaire. *Two-sided independent samples t-tests (df = 26), with equal variances assumed. †Two-sided independent samples t-test (df = 18.54), with equal variances not assumed. ‡P = 0.050.
Table 2. Means (±SD) of actigraphy and sleep diaries/group

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>SD Group</th>
<th>T value*</th>
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<tbody>
<tr>
<td><strong>Actigraphy</strong></td>
<td></td>
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<tr>
<td>Pre-experimental nights</td>
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<tr>
<td>TST</td>
<td>7.31 (±1.16)</td>
<td>6.89 (±0.98)</td>
<td>0.55</td>
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<tr>
<td>Bed time</td>
<td>0.27 (±1.08)</td>
<td>0.05 (±0.98)</td>
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<tr>
<td>Rising time</td>
<td>8.87 (±1.42)</td>
<td>8.21 (±1.35)</td>
<td>1.04</td>
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<tr>
<td>Experimental night</td>
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<tr>
<td>TST</td>
<td>6.46 (±0.86)</td>
<td>4.09 (±1.11)</td>
<td>6.05†</td>
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<tr>
<td>Bed time</td>
<td>0.83 (±0.59)</td>
<td>0.90 (±0.44)</td>
<td>0.33</td>
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<tr>
<td>Rising time</td>
<td>7.65 (±0.61)</td>
<td>4.57 (±1.73)</td>
<td>6.19†</td>
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<td><strong>Sleep diary</strong></td>
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<tr>
<td>Pre-experimental nights</td>
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<tr>
<td>TST</td>
<td>8.05 (±0.66)</td>
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<td>Bed time</td>
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<tr>
<td>Rising time</td>
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<td>9.02 (±1.46)</td>
<td>0.25</td>
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<tr>
<td>Experimental night</td>
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<tr>
<td>TST</td>
<td>7.23 (±0.94)</td>
<td>3.79 (±0.32)</td>
<td>12.98‡‡</td>
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<tr>
<td>Bed time</td>
<td>1.08 (±1.01)</td>
<td>0.48 (±1.05)</td>
<td>0.76</td>
</tr>
<tr>
<td>Rising time</td>
<td>7.66 (±1.14)</td>
<td>4.46 (±0.99)</td>
<td>7.76*</td>
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</table>

TST, total sleep time. Bed times and rising times are provided in fraction of 1 h (e.g., 12:30 AM = 0.50, and 11:45 PM = −0.25)* Two-sided independent samples t-test with equal variances assumed (df = 26). † P < 0.001. ‡ Two-sided independent samples t-test with equal variances not assumed (df = 17.5).

the main effect of group and the interaction did not reach statistical significance (all \( F \leq 1.5 \), all \( P > 0.10 \)).

**Behavioral Ratings**

All but one participant rated the shock probability for the CS\(^+\) higher than for the CS\(^-\). Twenty-eight out of 31 participants reported a difference of >15 percentage points between stimuli (true difference = 33 percentage points). To evaluate whether unawareness of stimulus contingencies affected the results, we re-ran the critical SCR and fMRI analyses without the three unaware participants (see below). The two-way (group repeated over stimulus) ANOVA of shock probability estimates resulted in a significant main effect of stimulus \( F_{(1,26)} = 72.46, P < 0.001 \); mean for the CS\(^+\) (\( M_{\text{CS}^+} \)) = 48.8 (std. dev. = 22.1) and mean for the CS\(^-\) (\( M_{\text{CS}^-} \)) = 11.4 (std. dev. = 12.8). The main effect of group and the group \( \times \) stimulus interaction was not significant (all \( F \leq 0.12 \), all \( P > 0.10 \)) Valence ratings mirrored these results: there were significant conditioning effects \( F_{(1,26)} = 21.28, P < 0.001 \); \( M_{\text{CS}^+} = 41.5 \) (std. dev. = 21.8) and \( M_{\text{CS}^-} = 19.0 \) (std. dev. = 21.0) but no main effects or interaction of group (all \( F \leq 0.16 \), all \( P > 0.10 \)).

**SCR and Pulse**

The three-way (group repeated over stimulus and time) ANOVA, conducted on the FR, yielded a significant stimulus \( \times \) time interaction \( F_{(3,78)} = 5.22, P < 0.005 \); no other effects or interaction terms reached statistical significance (all \( F \leq 1.37 \), all \( P > 0.10 \)). The interaction was due to a larger FR to the CS\(^+\) than to the CS\(^-\) on the first three trials (FR\(^+\)) over the whole group \( r_{(27)} = 3.32, P < 0.005 \). The exclusion of the three unaware subjects did not change these results. As before, only the stimulus \( \times \) time interaction reached significance \( F_{(3,69)} = 5.65, P < 0.005 \). The four-way (group repeated over stimulus, response window, and time) ANOVA performed on the first and last three trials of each response window (FR\(^+\), SI\(^+\), SI\(^-\), SI\(^-\)) led to a significant main effect of stimulus \( F_{(1,26)} = 10.60, P < 0.005 \) and a significant stimulus \( \times \) response window \( \times \) time \( \times \) group interaction \( F_{(1,53,39,84)} = 4.40, P < 0.05 \). This latter result implies that over time, the groups differed in their FRs, SI\(^+\), and SI\(^-\). However, the effects seem to be of a more general nature, as indicated by the trends for an effect of response window \( F_{(1,29,33,63)} = 3.06, P = 0.080 \) and a time \( \times \) group interaction \( F_{(1,26)} = 3.77, P = 0.063 \); no other effects or interaction terms were significant (all \( F \leq 2.97 \), all \( P \geq 0.10 \)).

A two-way (group repeated over time) ANOVA confirmed that these general effects were also evident in the SCR to the shocks themselves. Whereas the main effect of group failed to reach significance \( F_{(1,26)} = 0.86, P > 0.10 \), there were trends for a significant main effect of time \( F_{(3,78)} = 2.70, P = 0.051 \) and a significant group \( \times \) time interaction \( F_{(3,78)} = 2.38, P = 0.076 \). To evaluate whether the interaction term was due to differential shock habituation, the percent decrease in SCR from the first and second to the third and fourth shock application was computed. An independent samples t-test on this measure revealed that participants in the control group habituated to a significantly greater degree to the shocks than participants in the SD group \( t_{(26)} = 2.12, P < 0.05 \); see Fig. 2A). The exclusion of the three unaware participants did not change this result \( t_{(23)} = 1.85, P < 0.05 \).

The three-way (group repeated over stimulus and time) ANOVA conducted on the pulse data yielded a significant main effect of group \( F_{(1,26)} = 5.24, P < 0.05 \). No other effects or interaction terms were significant (all \( F \leq 1.93 \), all \( P > 0.10 \)).

**EEG Vigilance Control**

The three-way (group repeated over time and channel) ANOVA, computed on the EEG vigilance data, yielded a significant main effect of channel \( F_{(1,88,35,79)} = 94.33, P < 0.001 \) and a trend for a significant main effect of time \( F_{(4,16,78,96)} = 2.40, P = 0.055 \). Importantly, the main effect of group, as well as all interaction terms with the factor group, did not reach statistical significance (all \( F \leq 1.60 \), all \( P > 0.10 \)). This indicates that participants were equally vigilant when learning the CS-US contingency, regardless of whether they were sleep deprived or not.

**Functional MRI**

The two-way (group repeated over stimulus) ANOVA revealed no main effect or interaction of group. The stimulus main effect was reflected in significant clusters of activity in the bilateral thalamus, brain stem, anterior cingulate cortex, insula, supramarginal gyrus, and the orbital and triangular parts of the inferior frontal gyrus (\( P_{\text{FWE}} < 0.05 \)).

The two-way (group repeated over stimulus) ANOVA of the time modulation also did not yield a significant group \( \times \) stimulus interaction. The main effect of stimulus time modulation led to a significant cluster of activity comprising the right precentral gyrus, supplementary motor area (SMA), and paracentral lobule (\( P_{\text{FWE}} < 0.05 \)). A directed group contrast (SD group > control group), which can be interpreted as a group \( \times \) time interaction, revealed a significant cluster in the ventral hypothalamus (\( P_{\text{FWE}} < 0.05 \), \( k = 53 \), \( t \) value of the peak voxel (\( t_{\text{peak}} \) = 4.56 (4, 0, −8)) and a trend for a significant cluster in

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the cerebellum \( P_{\text{FWE}} = 0.06, k = 61, t_{\text{peak}} = 4.83 \) \((8, -60, -21)\). The contrast estimates indicated that activity in the hypothalamus cluster increased in the SD group and decreased in the control group over time, regardless of stimulus type (see Fig. 2B).

Correlations between SCR and Hypothalamus Activity

To evaluate the relationship between the SCR and fMRI results, PCAs with varimax rotation were conducted on the SCR values (see Supplemental Table S1) and on the extracted contrast estimate values of the hypothalamus and the cerebellum (linear time modulation; see Table 3). Loadings of individual items on components with an eigenvalue greater than one are displayed. The analyses yielded two components for the fMRI data, six components for the SCR data (see Supplemental Tables S1 and S2 and Table 3 for the factor structures), and 12 computed crosscorrelations. SCR components were labeled as: responses to CS\(^+\) and CS\(^-\) in early trials (component 3), middle trials (component 1), and late trials (component 2); response to the first CS\(^+\) presentation, including to shock (component 4); response to other three shock trials (component 5); and a rest component of two late CS\(^-\) trials (component 6).

The correlation between SCR component 2 (which can be described as “SCR to late stimulus trials”) and fMRI component 2 (which can be described as “hypothalamus and cerebellum activity change in response to shocks”) reached Bonferroni-corrected significance \((r = 0.58, P < 0.001; \text{see Fig. } 3)\). The same correlation was observed in the control group \((r = 0.71, P < 0.01)\) and SD group \((r = 0.50, P < 0.05)\) separately (see Fig. 3).
Table 3. *fMRI*-rotated components

<table>
<thead>
<tr>
<th>Components</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS⁻ (hyp)</td>
<td>0.815</td>
<td>0.047</td>
</tr>
<tr>
<td>CS⁺US⁻ (hyp)</td>
<td>0.871</td>
<td>0.061</td>
</tr>
<tr>
<td>CS⁺US⁺ (hyp)</td>
<td>0.268</td>
<td>0.676</td>
</tr>
<tr>
<td>CS⁻ (cer)</td>
<td>0.565</td>
<td>0.492</td>
</tr>
<tr>
<td>CS⁺US⁻ (cer)</td>
<td>0.524</td>
<td>0.668</td>
</tr>
<tr>
<td>CS⁺US⁺ (cer)</td>
<td>-0.241</td>
<td>0.753</td>
</tr>
</tbody>
</table>

Loadings of individual items (>0.4) on components with an eigenvalue >1 are displayed in bold. *fMRI*, functional MRI; CS⁻, safety stimulus; hyp, hypothalamus; CS⁺US⁻, conditioned stimuli that were not followed by a shock [unconditioned stimulus (US)]; CS⁺US⁺, CS that were followed by a shock (US); cer, cerebellum.

Check for Chronotype Confound

To verify that the reported results were not attributable to group differences in chronotype, all analyses that yielded a group effect or interaction term were repeated with the D-MEQ chronotype scores as a covariate.

**SCR data.** A two-way (group repeated over time) ANOVA performed on the SCR to the shocks themselves now resulted in a significant group × time interaction \([F_{(1,25)} = 4.85, P < 0.05]\). The four-way (group repeated over stimulus, response window, and time) ANOVA, conducted on the first and last three trials of each SCR window, still yielded a main effect of response window \([F_{(1,12,32.95)} = 3.94, P < 0.05]\) and a trend for a significant stimulus × response window × time × group interaction \([F_{(1,53,38.15)} = 3.53, P = 0.051]\). Any other effects and interaction terms failed to reach statistical significance (all \(F \leq 2.51, \alpha_{0.05} > 0.10\)).

**fMRI data.** When chronotype was entered as a covariate in the two-way (group repeated over stimulus) ANOVA on the linear time modulation of the stimulus regressors, the post hoc-directed group contrast (SD group > control group) revealed the same significant cluster of activation in the ventral hypothalamus \(P_{FWE} < 0.05, k = 54, t_{peak} = 4.63 (4, 0, −8)\).

The exclusion of the three unaware subjects did not change this outcome \(P_{FWE} < 0.05, k = 71, t_{peak} = 4.89 (4, 0, −8)\).

In addition, a flexible factorial ANOVA on the linear time modulation of the stimulus regressors was computed to control for any further potential influences of single-subject variance and the in-/exclusion of interactions. Here, the main effect of group revealed an even larger ventral hypothalamus cluster around the same peak voxel \(P_{FWE} < 0.001, k = 149, F\) value of the peak voxel \(F_{peak} = 34.97 (4, 0, −8)\). This analysis further revealed three clusters in the cerebellum, whereas additional clusters were noted in bilateral insula, middle cingulate cortex and SMA, inferior frontal gyrus (orbital part), superior temporal pole, and rolandic operculum (all \(P_{FWE} < 0.05\)).

**DISCUSSION**

This combined SCR/EEG/fMRI study investigated the effect of one night of partial SD on fear acquisition on two levels: 1) nonassociative learning processes, mediated by hypothalamus and brain stem, and 2) associative learning—differential fear conditioning—mediated by amygdala and mPFC.

**Effects of Partial SD on Nonassociative Learning Processes**

Our data clearly show that one night of partial SD affected nonassociative learning processes at the physiological level, manifest in a lack of habituation of SCR and hypothalamus activity during fear conditioning in the SD group. Moreover, we observed that increased hypothalamic and cerebellar activity was positively correlated with SCRs to late stimulus trials in both groups. This suggests a fundamental change of sympathetic responsiveness to stimuli after partial SD. Importantly, group differences were not confounded by chronotype, and objective vigilance, as measured with EEG, did not correlate with hypothalamus activity.

The hypothalamus is considered the main control center of sweat secretion (Boucsein 2012) and plays an integral role in the regulation of the sleep-wake cycle (Pace-Schott and Hobson 2002). It mediates the physiological footprint of the stress response via multiple pathways. One of them involves neuronal projections to the sympathetic nervous system in the spinal cord (Boucsein 2012). Another one involves the hypothalamus-pituitary-adrenal (HPA) axis and comprises a series of neuroendocrine steps that starts in the paraventricular nucleus of the hypothalamus and triggers the adrenal cortex to release glucocorticoids, such as cortisol, into the bloodstream (de Kloet et al. 2005). In healthy humans, cortisol secretion adheres to a 24-h profile with a steep increase during the second half of the night, high levels in the early morning, and a progressive decline throughout the rest of the day (Balbo et al. 2010). It is conceivable that SD critically changes the timing and output intensity of this neuroendocrine fluctuation. Until now, experimental effects of SD on cortisol levels have been ambiguous, with some studies reporting no changes after SD and others observing a mild increase or decrease of cortisol levels (Meerlo et al. 2008). In any case, it has been shown that the anticipation of sleep coming to an early end induces a forward shift in the rise and morning peak of HPA activity (Born et al. 1999). This lends support to the assumption that waking up 4 h before the normal rising time may have shifted daily cortisol fluctuation in the present study. Consequently,

**Fig. 3. Correlation between functional MRI (fMRI) and SCR components within and across experimental groups (r = 0.58, P < 0.001).** The relevant SCR component can be described as SCR responses to late stimulus trials, whereas the fMRI component reflects a mixture of hypothalamus and cerebellum activity increase, primarily (but not exclusively) to shocks.
the SD group could have presented with different cortisol levels than the control group at the 9 AM testing session.

In general, there is accumulating evidence that elevated cortisol levels are associated with stronger differential fear conditioning (Grillon et al. 2006; Pineles et al. 2013; Zorawski et al. 2005, 2006). Several fMRI studies have reported sex differences with a positive correlation between exogenously applied cortisol and differential BOLD responses for the CS+ > CS− contrast in women and a negative correlation in men (Merz et al. 2010; Stark et al. 2006; Tabbert et al. 2010). Pineles et al. (2013) reported a positive relationship between waking (saliency) cortisol level and the acquisition of conditioned fear in police cadets and firefighters. If SD would affect cortisol levels, this should, in turn, affect fear acquisition, but the effect of SD on fear acquisition was not significant in our data (see Effects of Partial SD on Associative Learning Processes).

Interestingly, Pineles et al. (2013) also found waking cortisol levels to be positively related to measures of physiological reactivity, including slower SCR habituation to loud tones. This is in line with SD resulting in more general physiological effects, such as the reduced SCR habituation in our study. However, as we did not measure cortisol levels, we can only conclude that the close link between sleep and HPA axis activity is likely to have contributed to the observed effects. What can be said is that our data do not show generally increased levels of activity in the hypothalamus but rather, a differential temporal pattern of hypothalamic reactivity to stimuli after SD, i.e., a lack of habituation.

Participants in the SD group were deprived of the second half of the night, resulting in a sleep manipulation more targeted at REM sleep than at slow-wave sleep. In this context, elevated levels of noradrenaline (NA) might have mediated increased hypothalamic activity during fear conditioning. The locus coeruleus (LC) is the main NA provider of the central nervous system and has been shown to promote wakefulness by activating noradrenergic β- and α1-receptors, expressed, amongst others, in the lateral hypothalamus (Berridge et al. 2012). The LC also contains the noradrenergic REM-OFF neurons that inhibit REM sleep generation (Pal and Mallick 2007). Except during REM sleep, these neurons are active in all stages (Mallick et al. 2009) and keep firing during REM SD (Mallick et al. 1990), which has been argued to induce NA in the brain after REM SD (Mallick and Singh 2011). This notion is supported by animal research, showing that REM SD decreased levels of monoamine oxidase-A, an NA-degrading enzyme, and increased levels of tyrosine hydroxylase, the first-rate limiting enzyme for NA synthesis (Majumdar and Mallick 2003; Thakkar and Mallick 1993). Further animal research demonstrated that REM SD influences brain excitability by increasing the activity of sodium-potassium ATPase and rendering the neuronal membrane potential more positive, a sign for enhanced excitation (Das and Mallick 2008; Gulyani and Mallick 1993). This effect was shown to be mediated by NA acting on the α1-adrenoceptor (Gulyani and Mallick 1995; Mallick and Adya 1999). Thus an elevation of NA after SD could well have led to increased excitability of the hypothalamus and counteracted habituation during fear conditioning in the SD group. One issue is that increased NA levels should have also been reflected in heightened vigilance. However, the EEG data indicate that vigilance levels and fluctuations did not differ significantly between groups.

Previous work has shown that electrical stimulation of the hypothalamus results in panic attack-like behavior in cats (Wheatley 1944), rats (Lammers et al. 1988), and according to a recent case report, in humans as well (Wilent et al. 2010). Interestingly, stimulation in the middle of the ventromedial hypothalamus elicited the most robust panic-like responses, consisting of hyperventilation, increases in blood pressure and heart rate, and subjective emotionality (Wilent et al. 2010). Our fMRI data not only revealed an effect of SD on hypothalamus activity but when controlling for covariates, also on the temporal pattern of activity in regions typically associated with saliency and affective processing, such as the SMA, rolandic operculum and bilateral insula, as well as middle cingulate and lateral orbitofrontal cortex [for a review on the role of these regions in anxiety-related processes, see Sylvester et al. (2012)]. To our knowledge, our study provides the first formal experimental evidence in humans that in the context of stressful events (i.e., fear conditioning), sleep effects and physiological-affective effects converge in the hypothalamus.

Effects of Partial SD on Associative Learning Processes

The effects of SD on amygdala-mediated associative learning processes were weaker than on nonassociative learning processes and did not reach statistical significance in our sample. The amygdala is considered integral to the acquisition and expression of conditioned fear (Phelps and LeDoux 2005), and one night of total SD was shown to decrease functional connectivity between mPFC and amygdala (Yoo et al. 2007). Moreover, SD has detrimental effects on emotional memory encoding (Walker 2008) and consolidation reliant on hippocampus and mPFC (Sterpenich et al. 2007). Emotional memory consolidation has been associated with REM sleep (Nishida et al. 2009; Walker and van der Helm 2009), whereas REM SD is related to amplified activity in areas central to emotional processing, including the mPFC (Rosales-Lagarde et al. 2012). The lack of SCR or fMRI effects in our study does not preclude that our experimental manipulation may have more long-term memory-encoding or consolidation effects. It has been observed that emotionally arousing experiences are more robustly encoded in memory than neutral ones, presumably because of the autonomic neurochemical reactions evoked at the time of the experience (McGaugh 2004). A next step would be to address whether fear-conditioning memory is encoded or consolidated differently after partial SD.

Limitations

One limitation of our data is that the effect of one night of partial SD on fear conditioning could have been too small to be detected. Stronger effects may be achieved by total SD, which has been shown to be a useful paradigm for investigating the neurocognitive effects of SD but rarely occurs in everyday life (Goel et al. 2009) and more strongly affects vigilance. Chronic sleep restriction may constitute a more ecologically valid, alternative intervention, but the dropout rates typically associated with such an intense procedure would probably be too high in the context of the complex and multimodal measurements used here. Nonetheless, it should be noted that the effect of one night of partial SD on a nonassociative learning process, such as physiological habituation, was much larger than any effect on the associative processes involved in conditioned learning.
fear. A further limitation of this study concerns the sample size, which was rather small for the statistical analyses of the behavioral and physiological data. Due to our multimodal approach, measurements were technically challenging, and data from four subjects had to be excluded, resulting in a sample size of 28 (medium sized for fMRI studies). Yet, despite this shortcoming, we observed robust fMRI effects.

Conclusion

Taken together, the presented results suggest that one night of partial SD affects activity in the hypothalamus and leads to altered peripheral physiological reactivity. Thus partial SD seems to have more pronounced effects on a basic physiological level than on associative learning. These findings add to our understanding of the potentially bidirectional relationship between sleep disturbances and anxiety.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.C.P. and V.I.S. conceived and designed research; A.C.P. and I.E. performed experiments; A.C.P. and V.I.S. analyzed data; A.C.P. and V.I.S. interpreted results of experiments; A.C.P. and V.I.S. drafted manuscript; A.C.P. and V.I.S. revised manuscript; A.C.P., J.B., P.G.S., M.C., and V.I.S. edited and revised manuscript; A.C.P., J.B., P.G.S., I.E., M.C., and V.I.S. approved final version of manuscript.

DISCLOSURES

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