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Sleep Debt Elicits Negative Emotional Reaction through Diminished Amygdala-Anterior Cingulate Functional Connectivity

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Abstract

Objectives: Sleep debt reportedly increases emotional instability, such as anxiety and confusion, in addition to sleepiness and psychomotor impairment. However, the neural basis of emotional instability due to sleep debt has yet to be elucidated. This study investigated changes in emotional responses that are elicited by the simulation of short-term sleep loss and the brain regions responsible for these changes.

Subjects and Methods: Fourteen healthy adult men aged 24.1±3.3 years (range, 20–32 years) participated in a within-subject crossover study consisting of 5-day sessions of both sleep debt (4 h for time in bed) and sleep control (8 h for time in bed). On the last day of each session, participants underwent polysomnography and completed the State-Trait Anxiety Inventory and Profile of Mood States questionnaires. In addition, functional magnetic resonance imaging was conducted while performing an emotional face viewing task.

Results: Restricted sleep over the 5-day period increased the activity of the left amygdala in response to the facial expression of fear, whereas a happy facial expression did not change the activity. Restricted sleep also resulted in a significant decrease in the functional connectivity between the amygdala and the ventral anterior cingulate cortex (vACC) in proportion to the degree of sleep debt (as indicated by the percentage of slow wave sleep and δ wave power). This decrease was significantly correlated with activation of the left amygdala and deterioration of subjective mood state.

Conclusion: The results of this study suggest that continuous and accumulating sleep debt that can be experienced in everyday life can downregulate the functional suppression of the amygdala by the vACC and consequently enhance the response of the amygdala to negative emotional stimuli. Such functional alteration in emotional control may, in part, be attributed to the neural basis of emotional instability during sleep debt.

Introduction

Many people are now suffering from chronic sleep loss as a result of today's 24-h society, night-owl lifestyles, and prolonged work hours becoming a normal state of everyday life [1,2,3,4,5,6]. Sleep loss causes day-time sleepiness and psychomotor impairment, and can result in human errors and accidents [7,8,9].

Acute sleep deprivation has been shown to augment physiological and psychological reactions to emotional stimuli. For example, compared with normal sleep conditions, overnight total sleep deprivation enhances sympathetic reactions to unpleasant stimuli, such as dilation of the pupils and increased heart rate and blood pressure [10,11], declined task performance due to increased

interference of working memory by unpleasant emotional stimuli [12], and increased changes in mood deterioration triggered even by weak emotional stressors [13]. According to functional brain imaging studies investigating the neural basis of emotional responses after acute sleep deprivation, unpleasant emotional stimuli increase the activity of the amygdala after overnight total sleep deprivation, suggesting a decline in functional connectivity between the amygdala and the medial prefrontal cortex (mPFC) which may reflect decreased inhibition by the frontal lobe [12,14].

In addition, Swann et al. have shown that response time to subliminal priming is shortened after having short hours of sleep over a 2-day period [15]. Because subliminal visual information is transmitted to the amygdala without going through the visual
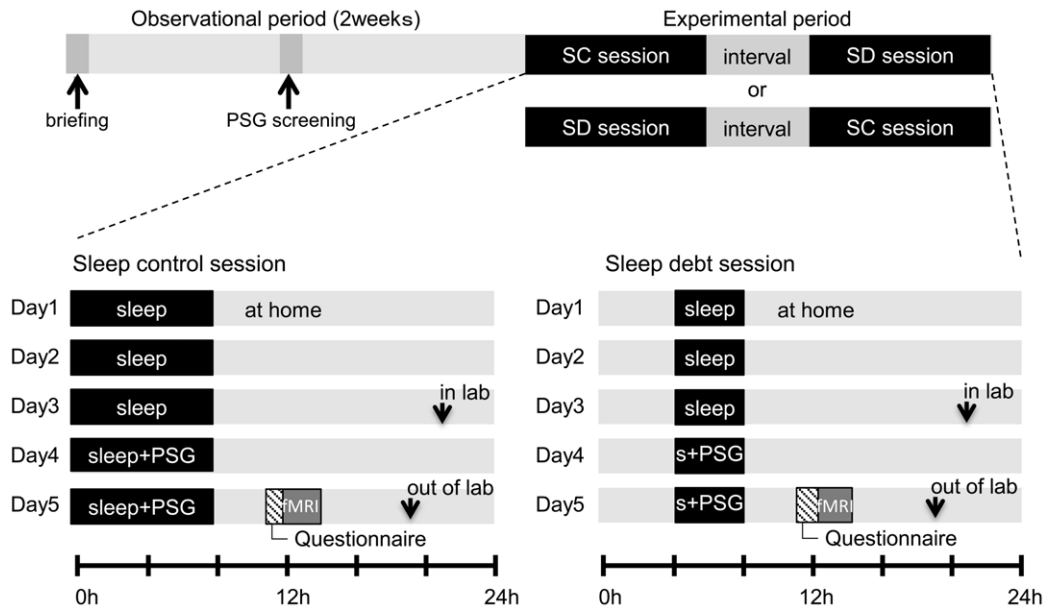
Figure 1. Experimental protocol. The study was conducted in a randomized crossover design, involving a sleep control (SC) and sleep debt (SD) session (for 5 days in each session) with a 2-week interval between the sessions. In the observational session before the experiment, participants visited the laboratory for a briefing session and gave their informed consent. One week later, participants came to the lab for PSG screening. One week after the PSG screening, the experimental sessions were started, with the order of the sessions counter-balanced across participants (i.e., SC-SD or SD-SC). Participants stayed at home on days 1–3 within each SC and SD session, according to the restrictive sleep-wake schedule that had been already instructed in the briefing (i.e., sleep time of 8 h for SC and 4 h for SD). Participants came to the lab on night 3 of the SC and SC sessions and spent the rest of the sessions (i.e., 2 days per session) in the sleep-lab with their sleep time controlled as instructed. On nights 3 and 4 in each session, participants underwent PSG measurement in the lab. On day 5, they completed questionnaires to check their mood state and sleepiness followed by fMRI scanning with an emotional task. SC, sleep control; SD, sleep debt; PSG, polysomnography; SSS, Stanford Sleepiness Scale; STAI, State-Trait Anxiety Inventory; POMS, Profile of Mood States.
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cortex [16,17,18,19,20] and subliminal and supraliminal stimulation induce different responses in the amygdala [21,22], it is possible that the transduction through subliminal signal pathways may also play an important role in changes in emotional responses to visual stimuli after sleep deprivation.

On the other hand, deteriorated mental and physical conditions due to sleep loss (partial sleep deprivation) are more likely to be caused by an accumulation of short sleep episodes over several days (sleep debt) than overnight total sleep deprivation [23]. Although sleep debt reportedly augments emotional instability (including anxiety and confusion), together with sleepiness, the feeling of fatigue, and deficits in psychomotor performance [24,25,26,27,28], the characteristics of emotional responses induced by sleep debt and the neural basis underlying such responses have not been studied extensively and therefore remain unclear.

In this study, we simulated continuous and accumulating sleep debt that can be experienced in everyday life (short hours of sleep over a 5-day period) to investigate changes in emotional responses caused by visual stimuli presented above and below the level of consciousness and the brain regions responsible for these changes.

Materials and Methods

Ethics Statement

This study was approved by the Ethics Committee of the National Center of Neurology and Psychiatry, Japan and was conducted in accordance with the Declaration of Helsinki.

Participants

This study involved 14 healthy, right-handed adult men (mean ± SD age, 24.1±3.32 years) who provided written informed consent to participate. All participants were Japanese and native Japanese speakers. A sleep log and actigraph (Ambulatory Monitoring Inc., Ardsley, NY) were used to monitor the sleep schedule of participants during the observational period (a 2-week period prior to the study) and the following experimental period. Using Cole's algorithm with optimal parameters [29], sleep-onset time, wake time, and the amount of time awake in bed were calculated from the actigraph data and were compared with the sleep log to confirm the absence of irregular life patterns, such as working in shifts or staying up all night. Overnight polysomnography (PSG) was also conducted during the observational period to examine for sleep disorders.

Exclusion criteria were as follows: a mean bedtime or wakeup time during the observational period outside of the hours 23:00–02:00 and 07:00–10:00, respectively(including shift worker); some form of sleep disorder; serious physical complication; psychiatric disorder; ocular disease, including achromatopsia; taking medication or substances inveterately that might affect the experimental data (e.g., steroids and drugs that induce drowsiness such as hypnotics and anti-histamines); caffeine intake of over 200 mg per day, heavy smoker (stressed by a 5-day smoking cessation) implanted metal object such as a pacemaker; working shifts (engaged in shift work in the 4 weeks preceding the study); or travelling to a country with a 6-h time difference in the 3 months preceding the study.

Sleep restriction protocol

Figure 1 shows the experimental protocol. All participants attended the briefing session concerning the experimental outline, underwent sleep electroencephalography (EEG) screening during the 2-week observational period, and participated in two 5-day experimental sessions. The number of hours in bed (i.e., after lights-out and during sleep) was 8 h/day in the sleep control (SC) session and 4 h in the sleep debt (SD) session. Both sessions were conducted as a crossover study with a 2-week interval between the sessions. During the interval, participants were asked to maintain a regular lifestyle without staying up all night or taking shift work.

In the SC session, based on the sleep log and actigram from the observation period, mean bedtime (23:00–02:00) for each individual was used as the start time for 8 h of sleep (wakeup time 07:00–10:00). In the SD session, bedtime started 4 h later (03:00–06:00) than that in the SC session and total hours in bed were 4 h (wakeup time 07:00–10:00).

In both sessions, participants stayed home for the first 3 days and then stayed in a laboratory room at the National Center of Neurology and Psychiatry for the next 2 days. To maintain the strict wakeup time at home, we sent a mail alert every 4 h, starting at the scheduled wakeup time until bedtime, and asked participants to answer the mail immediately. Participants were instructed by E-mail to refrain from caffeine and alcohol intake and smoking during the 5 days in which the sessions were held. In the laboratory, participants were under video camera surveillance, always assisted by a research attendant, and verbally awakened when in a drowsy state, such taking a nap or dozing off. During the wake period, participants were allowed to move freely around the laboratory, read and write, enjoy music and videos, play videogames, and engage in conversation with a researcher. Mineral water was always available, but the intake of caffeine and alcohol, smoking, and heavy exercise were restricted. Ambient temperature and humidity in the laboratory were maintained at $25\pm0.5^{\circ}C$ and $50\pm5\%$ RH, respectively.

MRI and emotional face viewing task

Magnetic resonance imaging (MRI) was performed on day 5 of the sessions. Participants were first served the same breakfast (~350-kcal sandwich) within 2 h of wakeup time before entering a room next to the MRI room 2–2.5 h after the wakeup time to answer a questionnaire about subjective sleepiness and mood. They underwent MRI 3–5 h after wakeup time.

During MRI, participants viewed faces with emotional expressions under two different conditions: (1) the conscious condition with a sufficient viewing time to allow supraliminal visual perception of an emotional facial expression and (2) the non-conscious condition with a brief viewing time to perform subliminal perception of an emotional facial expression followed by a neutral facial expression to mask the emotional facial image (Fig. 2).

We selected portraits of 16 individuals (4 Caucasian men, 4 Caucasian women, 4 Japanese men, and 4 Japanese women) from two standardized image sets ([30,31]www.atr-p.com/face-db.html) and created 3 facial images per individual representing the categories of fear, happy, and neutral facial expressions by masking the hair and background (48 images in total).

(1) Under the conscious condition, a fixation image was presented for 1000 ms, followed by one of the three types of facial expressions for 200 ms and then a blank image for 1000 ms. (2) Under the non-conscious condition, after presenting a fixation image for 1000 ms, either (i) a neutral facial image was presented for 26 ms, followed by a neutral facial image of another person of the same sex for 174 ms and then a blank image for 1000 ms, or

(ii) a happy (fear) facial image was presented for 26 ms, followed by a neutral facial image of the same person for 174 ms and then a blank image for 1000 ms.

In both the conscious and non-conscious presentations, therefore, one trial consisted of the presentations of a fixation image, a facial image in the conscious condition or two images in the non-conscious condition, and a blank image. Nine trials composed one block. Among each block of 9 trials, a target stimulus was presented randomly, to which participants were to respond by pressing a button in order to keep themselves awake and focused on the images. After completing each block, a fixation image was shown on the screen for 15 s (baseline). A total of 12 image presentation blocks (i.e., one session) were conducted under the non-conscious and conscious conditions (6 blocks each) with a 15-s baseline period provided every 2 blocks, and a total of 2 sessions were performed with a 2-min break between the sessions. The order of block presentation was counter-balanced between participants and sessions.

Questionnaire

The Stanford Sleepiness Scale (SSS; [32]) was used to assess subjective sleepiness. Subjective mood was evaluated using state components of the State-Trait Anxiety Inventory (STAI-S; [33]), as well as the Profile of Mood States (POMS; [34]). During the SC and SD sessions, participants answered the questionnaire immediately prior to MRI.

Polysomnography (PSG) and delta wave power analysis

On nights 4 and 5 of each session, PSG was performed and analyzed using the Neurofax EEG-1200 (Nihon Kohden Corporation, Tokyo, Japan) with Ag/AgCl electrodes. The system recorded an electrocardiogram (ECG), electrooculogram (EOG), electromyogram of the chin (Chin-EMG), and electroencephalogram (EEG) at C3, C4, O1, and O2 sites in line with the International 10–20 system using the mastoid processes as reference points. The sampling rate was 200 Hz, and the hardware bandpass filter was set at 0.5–35 Hz. EEG recordings from C3-A2 were used to perform visual classification of sleep stages in 30-s epochs in accordance with international sleep scoring parameters [35].

The PSG data obtained on night 4 was excluded from the analysis to eliminate the first night effect [36]. The following sleep parameters were calculated from the PSG data taken on night 5: total sleep time (TST), duration of each sleep stage, duration of slow wave sleep (Stage 3+4; SWS) during the 2-h period after bedtime, sleep latency (SL), sleep efficiency (SE), percentage of SWS spent in TST (%SWS), and percentage of rapid eye movement (REM) sleep (%REM).

Because sleep debt increases the duration of SWS and delta power during the initial stage of sleep [27,37,38,39,40], during the first 2 h after bedtime the amounts of SWS ($SWS_{2 h}$) and δ power ($\delta_{2 h}$, 0.5–4 Hz) were used as objective indicators of sleep debt.

After visually excluding epochs containing body movement artifacts, all NREM sleep epochs (Stages 2–4) in the C3 EEG recording were analyzed using the fast Fourier transform (5.12-s hamming window, 2.5-s steps), and based on the power values obtained every 0.2 Hz, δ power (0.5–4 Hz) was calculated.

fMRI acquisition

The Siemens Magnetom Verio 3T MRI system was used in the analysis. To obtain reference images for analysis, structural images (T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) images) were taken with the following sequence
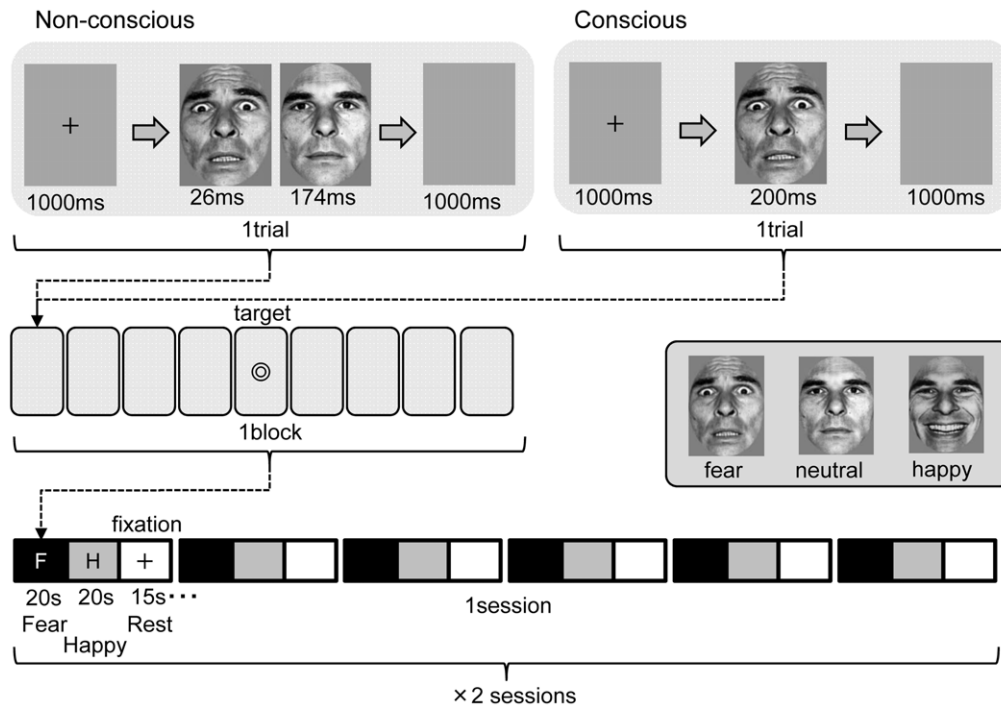
Figure 2. Design of emotional facial presentations. Facial pictures depicting fear or happy (i.e., emotional) or neutral expressions were used as the stimuli and were presented either non-consciously or consciously. In a non-conscious trial, an emotional image (either fear, happy, or neutral) was implicitly presented for 26 ms, followed by an explicit presentation for 174 ms of a neutral 'masking' face of the same identity as the preceding implicit emotional face (when the implicit face was neutral, the following explicit mask was of a different person of the same sex). Participants were required to press a button in response to each 'target' stimulus to keep themselves awake during the scanning.
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parameters: TR/TE = 1900/2.52 ms, voxel size = 1×1×1 mm, flip angle 9°, and field of view = 256×192 mm.

A single shot echo-planar imaging technique was used to obtain task-related functional MRI (fMRI) images. Settings were: TR/TE = 2500/25 ms, 30 axial slices, voxel size = 3×3×4 mm, 1-mm inter slice gap, flip angle 90°, matrix size = 64×64, and field of view = 192×192 mm. Of 137 scanning images obtained in each session, the first 5 images were excluded from analysis.

fMRI data analysis

SPM8 (Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) was used in the analysis of functional brain imaging data. For each image, motion and slice timing correction as well as co-registration into an MPRAGE structural image was performed. MPRAGE imaging was carried out after PSG screening. The Montreal Neurological Institute (MNI) template was used for spatial normalization, and smoothing was performed using an 8-mm full width of half maximum Gaussian kernel. MRI time-series data that contained the three-dimensional blood oxygenation level dependent (BOLD) signals of each participant were analyzed using the first-level fixed effect model with general linear model (GLM) regression analysis. Using the canonical hemodynamic response model implemented in SPM8, a hypothetical hemodynamic time course corresponding to the stimulus presentations under each task condition was developed by convolving the canonical function. Thirteen hemodynamic models of time series corresponding to i) 6 conditions [3 categories of emotions (happy, fear, and neutral)×2 types of image presentation (conscious/non-conscious)], ii) target image presentation, and iii) 6 head motions as regressors were incorporated into the design matrix. Actual BOLD signals were analyzed voxel by voxel using the GLM, and the parameter estimate for each regressor was calculated. To subtract the low visual features, contrasts were created by subtracting the activity at the time of neutral facial image presentation from the activity at the time of emotional facial image presentation. Consequently, a total of 4 contrasts for a fear vs. neutral facial image and happy vs. neutral facial image under the conscious and non-conscious conditions were created.

To determine differences between the SC and SD sleep conditions, the value of the first-level contrast images in each SC and SD session were entered into a paired t-test implemented in SPM, with SC/SD as a within-subject factor.

Based on the hypothesis that sleep debt enhances the activity of the amygdala [14], we set the amygdala as the region of interest (ROI) and searched for the area where the activation was higher during the SD session than the SC session. Using the PickAtlas software (Wake Forest University (WFU), http://fmri.wfubmc.edu/downloads/WFU_PickAtlas_User_Manual.pdf) in the SPM Toolbox, masks for the amygdala on both sides were generated based on Anatomical Automatic Labeling (AAL), and each voxel in the mask was analyzed. Data were considered significant if p was less than 0.001 and the number of continuous voxels forming a cluster was greater than 5 within the amygdala ROIs. Furthermore, the significant cluster was corrected by family-wise error (FWE) correction within the amygdala ROIs (p<0.05, small volume correction [41]).

Functional connectivity analysis

To reveal the functional connectivity related to enhanced amygdala activation during sleep debt, a cluster in the left amygdala (peak MNI coordinates x = −14, y = 4, z = −18, 8

voxels, see Results) that exhibited a significant difference in fear vs. neutral contrast between the SD and SC sessions was used as the seed region of the connectivity analysis. Based on a previous study hypothesizing that sleep debt weakens the functional connectivity between the amygdala and the mPFC adjacent to the ACC [14], we placed the ROI in the mPFC region for the functional connectivity analysis and searched for the area that exhibited greater connectivity with the seed region in the SC session than that in the SD session. Using the WFU PickAtlas software in the SPM Toolbox, a mask for the ACC/mPFC region was generated based on AAL (by combining 'anterior cingulate' and 'medial frontal gyrus'), and each voxel in the mask was analyzed. The functional connectivity between the amygdala and the ACC/mPFC was analyzed using the CONN tool (Alfonso Nieto-Castanon, http://www.alfnie.com/software/conn) of SPM8.

Using GLM, voxels that were activated in relation to the BOLD signals in the seed region were extracted. Head motions and the hypothetical hemodynamic response to the main event (confounding effects of stimulus-locked transients [42]) and to the target were used as regressors, and the range of the bandpass-filter was set at 0.008–0.09 Hz. Connectivity contrasts thus created were used in first-level and second-level analyses, as in the analysis described earlier in the 'fMRI data analysis' section. Data were considered significant if p was less than 0.001 and the number of continuous voxels forming a cluster was greater than 5. Because of the larger size of the mask in the MPFC/ACC region, the small volume correction was not performed in functional connectivity analysis.

Correlation analysis between mood/sleep changes and fMRI data

Differences in the values between sessions were calculated for subjective mood (scores from the STAI-S and POMS questionnaires), $SWS_{2\,h}$, and $\delta_{2\,h}$. The contrast values between the sessions were also calculated for 1) amygdala activation and 2) the intensity of functional connectivity between the left amygdala and the ventral anterior cingulate cortex ($FC_{amg\text{-}vACC}$) in the fear vs. neutral condition under conscious presentation. Correlations between these psychometric and imaging contrast values were analyzed.

Clusters used in the analysis were the left amygdala, which showed differential activation between the SD and SC sessions, and the vACC, which showed different degrees of functional connectivity with the amygdala, as the seed region, between the sessions. Marsbar software (Matthew Brett, http://marsbar.sourceforge.net/marsbar.pdf) was used to calculate the mean contrast values in a cluster.

Statistics

The SPSS PASW Statistics 18 software package was used in statistical analysis. Differences in questionnaire scores, PSG data, and values of δ power between the SD and SC sessions were analyzed using the two-tailed t-test. Results are expressed as mean $\pm$ SD. Between-subjects test was performed by calculating Pearson's product moment correlation coefficient. Except for the analysis of functional brain activity, data were considered significant at $p<0.05$.

Results

Sleep time regulation

From the actigraph data, mean sleep time over the entire 5-day period in the SC and SD sessions was 8.09 ± 0.35 h (8 h 5 min $\pm$ 21 min) and 4.60 ± 0.54 h (4 h 36 min $\pm$ 32 min), with

significantly fewer sleep hours (3.48 ± 0.54 h, or 3 h 29 min $\pm$ 32 min) in the SD session [$t(13)=24.17$, $p<0.001$].

Sleepiness and mood states

Table 1 shows sleepiness and mood states associated with the SC and SD sessions. SSS and STAI-S scores for the SD session were significantly higher than those for the SC session, whereas no significant session-related differences were seen in POMS subscale scores.

PSG/delta wave power data

Sleep parameters and the analysis results are shown in Table 2. Compared with the SC session, the duration of TST, Stage 1, Stage 2, and REM were significantly shorter in the SD session; however, no differences were observed with Stage 3+4 or $SWS_{2\,h}$. As a result, the SD session had significantly higher %SWS and SE and significantly shorter SL. In addition, $\delta_{2\,h}$ for the SD session was significantly higher than that for the SC session.

Button Response

No significant session-related differences were seen in either the number or the mean time of responses (SC $=11.63\pm0.6$, SD $=11.38\pm1.16$, SC $=596.98\pm0.153.43$, SD $=608.28\pm115.34$, respectively).

fMRI activation

Comparison of fear vs. neutral contrasts for the conscious condition revealed significantly greater activation of the left amygdala in the SD session than in the SC session [peak MNI coordinates x $=-14$, y $=4$, z $=-18$, $t(13)=-5.60$, FWE $p<0.05$ small volume correction] (Fig. 3; Table 3). Even though the activation of the right amygdala in the SD session was also higher than that in the SC session, it did not reach the significance level set for the analysis [peak MNI coordinates x $=18$, y $=2$, z $=-18$, $t(13)=-3.41$, $p=0.002$]. With regard to happy vs. neutral contrasts, the amygdala showed no session-related differences in activation [left amygdala, x $=-14$, y $=4$, z $=-14$, $t(13)=-3.04$; right amygdala, x $=16$, y $=2$, z $=-16$, $t(13)=-1.90$].

Under the non-conscious condition, no significant differences in amygdala activation between the sessions was observed with fear vs. neutral contrasts [left amygdala; x $=-14$, y $=-8$, z $=-16$,

Table 1. Subjective sleepiness and mood state scores for the sleep control (SC) and sleep debt (SD) sessions; t and p-values for SC vs. SD with the paired t-test.

	SC session	SD session	t	p
SSS	2.14 (0.66)	3.21 (1.05)	−3.51	<0.01
STAI-state	35.64 (6.21)	39.43 (4.86)	−2.74	<0.05
POMS Vigor	53.54 (10.02)	53.54 (11.57)	0.00	N.S.
POMS Depression	47.76 (9.00)	48.13 (8.71)	−0.40	N.S.
POMS Anger-Hostility	42.84 (4.52)	42.24 (6.18)	0.60	N.S.
POMS Fatigue	45.94 (6.61)	48.13 (7.48)	−1.30	N.S.
POMS Tension-Anxiety	46.37 (10.57)	47.05 (9.92)	−0.46	N.S.
POMS Confusion	48.12 (8.07)	49.94 (9.32)	−0.99	N.S.

Data are expressed as mean (standard deviation) values; SSS, Stanford Sleepiness Scale; STAI, State-Trait Anxiety Inventory.
POMS, Profile of Mood States.
Degrees of freedom (df) = 13.
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Table 2. Values of spectral analysis and sleep variables for the sleep control (SC) and sleep debt (SD) sessions; t and p-values for SC vs. SD with the paired t-test.

	SC session	SD session	t	p
TST (min)	446.7 (22.5)	233.5 (7.5)	38.71	<0.001
Stage1 (min)	35.8 (20.0)	10.8 (8.2)	5.31	<0.001
Stage2 (min)	236.9 (35.3)	107.3 (28.9)	15.25	<0.001
Stage3 (min)	40.0 (11.3)	39.6 (15.4)	0.13	N.S.
Stage4 (min)	20.9 (25.8)	24.1 (25.8)	−1.52	N.S.
SWS (min)	60.8 (28.7)	63.7 (26.7)	−0.70	N.S.
REM (min)	113.1 (21.2)	51.7 (17.1)	11.09	<0.001
$SWS_{2\,h}$ (min)	37.5 (18.6)	41.4 (18.4)	−1.27	N.S.
Sleep latency (min)	19.3 (23.5)	3.3 (4.9)	2.90	<0.05
%SWS (%)	13.7 (6.7)	27.3 (11.6)	−7.21	<0.001
%REM (%)	25.4 (4.7)	22.2 (7.2)	1.80	N.S.
Sleep efficiency (%)	93.0 (4.7)	97.1 (3.1)	−3.46	<0.01
$Delta_{2\,h}$ (uV^2/min)	1340 (275)	1495 (312)	−3.108	<0.01

Data are expressed as mean (standard deviation) values; SC, sleep control; SD, sleep debt; SWS, slow wave sleep.
$SWS_{2\,h}$, slow wave sleep of first 2 h from sleep onset; $Delta_{2\,h}$, Delta wave power (0.5–4 Hz) of first 2 h from sleep onset.
Degrees of freedom (df) = 13.
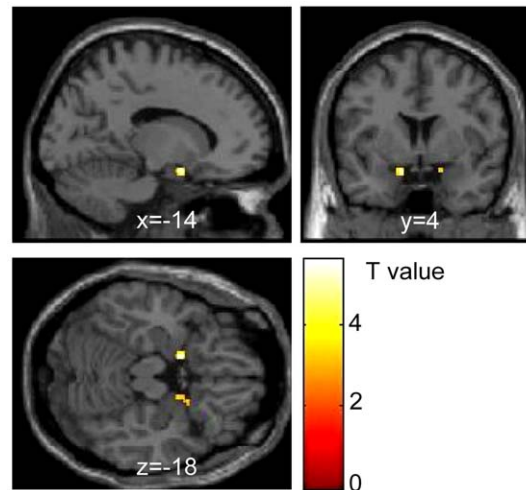doi:10.1371/journal.pone.0056578.t002

Figure 3. Difference in amygdala activation between the sleep control (SC) and sleep debt (SD) sessions. The map shows significantly greater activation in response to fearful face stimuli in the SD than SC session. Significant differences were seen in the left amygdala, peak MNI coordinate $(x, y, z) = (−14, 4, −18)$ mm, $T(13) = 5.60$, $p = .0001$, $k = 8$ contiguous voxels. A similar trend was also observed in the right amygdala, $(x, y, z) = (18, 2, −18)$ mm, $T(13) = 3.41$, $p = .0005$, $k = 7$. Significant clusters are rendered on a T1 anatomical referential image displayed in neurological convention, with the left side corresponding to the left hemisphere. The clusters shown are thresholded with a lenient alpha level ($p < 0.01$, $k > 5$) for visualization purposes. MNI, Montreal Neurological Institute template.
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$t(13) = −3.05$; right amygdala, x = 24, y = −4, z = −12, $t(13) = −3.08$] or happy vs. neutral contrasts [left amygdala; x = −22, y = −8, z = −14, $t(13) = −2.26$; right amygdala, x = 26, y = −6, z = −16, $t(13) = −2.60$].

We performed the analysis of functional connectivity using only the conscious and fear conditions that showed differential activation between the SD and SC sessions.

fMRI functional connectivity

Comparative analysis of fear vs. neutral contrasts for the conscious condition revealed that, compared with the SC session, $FC_{amg-vACC}$ was significantly diminished in the SD session [x = 14, y = 32, z = −4, $t(13) = 4.77$] (Fig. 4; Table 3).

Analysis of all of the task results from the SD and SC sessions showed a significantly negative correlation between the activation of left amygdala and $FC_{amg-vACC}$ [$r(13) = 0.63$, $p < 0.001$] (Fig. 5).

Correlations between mood/sleep changes and fMRI data

Sleep debt-related cross-correlation between left amygdala activation, $FC_{amg-vACC}$, and changes in mood and sleep states are shown in Table 4. Changes of $FC_{amg-vACC}$ between sessions ($\Delta FC_{amg-vACC}$) were negatively correlated with the changes of degree of sleep debt ($\Delta SWS_{2\,h}$ and $\Delta \delta_{2\,h}$) as well as mood changes (ΔSTAI-S (Fig. 6), ΔPOMS Tension-Anxiety, and ΔPOMS Confusion). On the other hand, no significant correlations were observed between Δamygdala activation and any of the parameters of the changes in mood or sleep states.

Discussion

The results of this study revealed that sleep debt caused by having just a few hours of sleep for 5 days (3 h 29 min/day of sleep restriction compared with the SC session) increased the activity of the left amygdala in response to a fear facial image. In contrast, a happy facial image did not change the activity. Functional connectivity analysis demonstrated that the levels of $FC_{amg-vACC}$ (left amygdala-vACC functional connectivity) were lower in participants with higher degrees of sleep debt ($\Delta SWS_{2\,h}$ and $\Delta \delta_{2\,h}$). The most important and novel finding in this paper is that declines in $FC_{amg-vACC}$ were correlated with left amygdala activation and subjective mood deterioration (higher STAI-S and POMS scores). These findings strongly suggest that down-regulation of the amygdala by the vACC and subsequent activation of the amygdala in response to negative emotional stimuli are involved in intensified physiological and psychological responses [10,11,12] and mood deterioration [13,28] due to unpleasant emotional stimuli during sleep debt.

This interpretation is supported by a series of studies. The amygdala is thought to play an important role in the expression of negative emotions [43,44,45]. Facial expressions of fear were found to activate the amygdala even in healthy individuals with normal sleep [46,47], and such activation is reportedly more prominent in individuals with depression and anxiety disorders [48,49,50]. Moreover, the amygdala has a strong functional and anatomical connection with the mPFC region [51], and the strength of this functional connection is correlated with the degree of subjective emotional suppression and reappraisal of negative affect. [12,52,53].

According to previous studies [12,14], overnight total sleep deprivation diminishes the functional connectivity between the amygdala and the mPFC. In the present study, even a short-term, continuous and accumulating sleep debt that can occur in everyday life clearly resulted in reduced functional connectivity between the amygdala and the mPFC, and more specifically the vACC.

Table 3. Anatomical coordinates for regions of significant difference between the sleep control and sleep deprivation session compared with fear vs. neutral contrast.

Area		BA	MNI x	y	z	t	Z	p	Cluster k*
SD>SC Activity in amygdala mask									
Left	Amygdala	34	−14	4	−18	5.6	3.93	<0.001	9
SC>SD Functional connectivity with amygdala in ACC/MPFC mask									
Right	Anterior Cingulate	32	14	32	−4	4.77	3.56	<0.001	9

SC, sleep control session; SD, sleep debt session.
Cluster k* $p<0.001$ uncorrected threshold.
Degrees of freedom (df) = 13.
doi:10.1371/journal.pone.0056578.t003

More importantly, subjective mood changes (increased anxiety) following short sleep were correlated with diminished $FC_{amg-vACC}$, but not with the change in the activation in the amygdala (Δamygdala activation) itself. This may indicate that diminished synchronization between the amygdala and the vACC plays a more important role than the extent of the event-related local activation in the amygdala, for stabilizing increased anxiety evoked by an unpleasant emotional stimulus. Some models of amygdala functionality [53,54] suggest that the magnitude of the local activity in the amygdala does not play a direct role in modulating the mood states of individuals, but the functional connectivity between the amygdala and the ventral mPFC correlates with STAI state score during resting state fMRI [55]. In our functional connectivity analysis, the main event-related hemodynamic response covaries with the seed-related (amygdala) activity in the GLM model of functional connectivity analysis; namely, main event-related immediate reactivity was regressed in our functional

connectivity results (see Friston et al., 1997 [42] for the detailed process). Therefore, $FC_{amg-vACC}$ in this study does not include simple event-related 'co-activation' between two regions, but finer event-unrelated synchronization between two regions beyond the local activities in two regions. This is why the local amygdala activity and the functional connectivity did not correlate with the psychological measurements in the same way; namely, the sustained anxiety (mood) state correlated with the $FC_{amg-vACC}$, but not with the event-related local reactivity of the amygdala. This interpretation is supported by the diminished functional connectivity between the amygdala and the vACC or ventral mPFC, regardless of the amygdala's activity, in individuals with social anxiety disorder, in those with the s allele of the serotonin transporter gene and who thus have a high risk of depression, and in those with schizophrenia [56,57,58].

Although it has been shown that positive emotional stimuli also activate the amygdala [54,55], happy facial expressions did not significantly alter amygdala activity during sleep debt in the present study. This suggests that functional changes in the amygdala and $FC_{amg-vACC}$ during sleep debt become more apparent when negative emotional stimuli are presented. Overnight total sleep deprivation reportedly induces activation of the
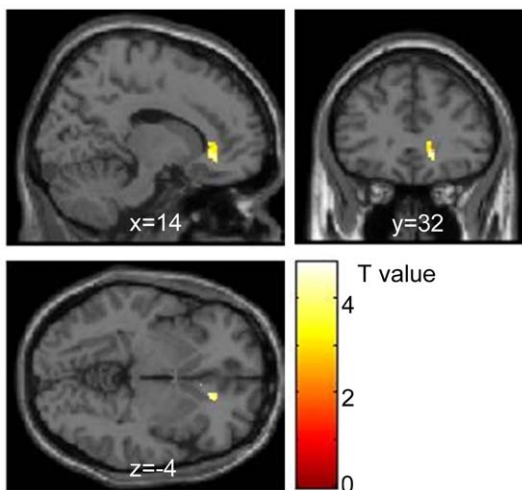

Figure 4. Difference in functional connectivity between sleep control (SC) and sleep debt (SD) sessions. The map shows greater functional connectivity between the left amygdala and other voxels in the brain in SC than SD session. Significant differences were found in the vACC, peak MNI coordinate (x, y, z) = (14, 32, −4) mm, T(13) = 4.77, p = .0001, k = 9 contiguous voxels. The significant cluster with a stronger connection with the left amygdala is rendered on a T1 anatomical referential image displayed in neurological convention, with the left side corresponding to the left hemisphere. The clusters shown are thresholded with a lenient alpha level (p<0.01, k>5) for visualization purposes. MNI, Montreal Neurological Institute template; vACC, ventral anterior cingulate cortex.
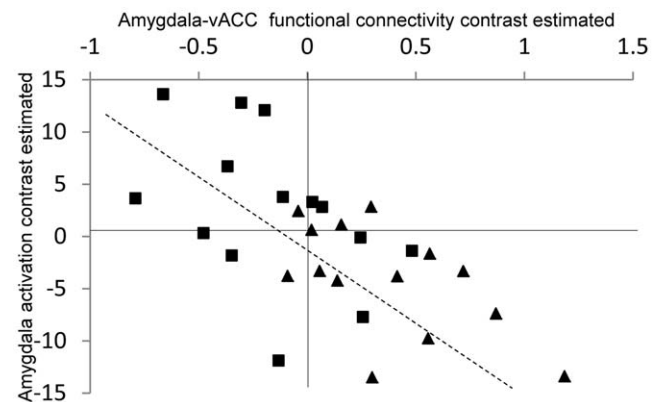doi:10.1371/journal.pone.0056578.g004

Figure 5. Correlation between amygdala activation and amygdala–vACC functional connectivity. Amygdala activation in response to fearful facial stimuli was negatively correlated with amygdala-vACC functional connectivity, r(13) = .64, p = .0001. The selected seed region within the amygdala was a cluster that showed greater functional connectivity with vACC in the SC than SD condition (p<0.001, uncorrected). Data from the SC and SD sessions were combined and plotted in one graph but differently colored; SD data in squares, SC in triangles. vACC, ventral anterior cingulate cortex; SC, sleep control condition; SD, sleep debt condition.
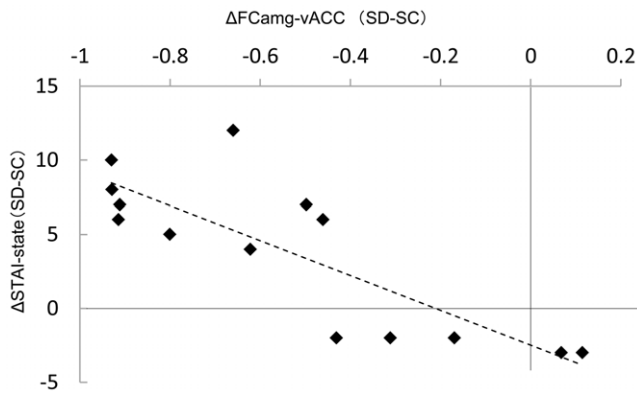doi:10.1371/journal.pone.0056578.g005

Figure 6. Correlation between the inter-session differences of amygdala–vACC functional connectivity and the inter-session differences STAI-state score. Inter-session differences between sleep control and sleep debt sessions of amygdala-vACC functional connectivity in response to fearful facial stimuli correlated negatively with inter-session differences of STAI-state score, $r(13) = .82$, $p = .0001$. Δvalue, inter-session difference between sleep control and sleep debt sessions for each value; vACC, ventral anterior cingulate cortex; SC, sleep control condition; SD, sleep debt condition; $FC_{amg-vACC}$, functional connectivity between amygdala and ventral ACC; STAI, State-Trait Anxiety Inventory.
doi:10.1371/journal.pone.0056578.g006

amygdala even in response to images associated with positive emotions [56]. It is reasonable to assume that this discrepancy is due to differences in the sleep conditions, such as total sleep deprivation in the former study and 5-day sleep restriction in the present one. It is possible that overnight sleep deprivation, more than accumulating sleep debt, affects the expression of positive emotion. In fact, overnight sleep deprivation elicits an antidepressant effect in patients with depression, as well as mood activation in healthy individuals [57,58,59,60,61]. Such effect and mood activation might be related to enhanced amygdala activation to positive emotional stimulus, as observed in a previous study. This notion is supported by the findings of a study in which antidepressant treatment enhanced amygdala activation in response to happy faces in patients with depression [62]. However, no similar phenomena have been reported in individuals with consecutive nights of sleep loss.

Unexpectedly, the presentation of emotional facial images under the non-conscious condition did not elicit changes in the amygdala activation after sleep restriction in the present study. A previous study showed that responses to masked priming are enhanced after a 2-day partial sleep deprivation [15]. Moreover, overnight total sleep deprivation strengthened the functional connectivity of amygdala with the sub-cortical region (midbrain) while diminishing the functional connectivity with the mPFC [14]. Based on these findings, we had expected to observe certain functional alteration in not only conscious processing through the cortical pathway, but also non-conscious processing through the subcortical pathway. However, no such alteration was observed in our participants. Any changes in non-conscious processing might be observable by adjusting the length of non-conscious image presentation, the intensity of the facial image stimuli, or increasing the sample size.

Interestingly, the decline in $FC_{amg-vACC}$ was correlated with the increase in %SWS and δ wave power in the early period of the sleep, but not with subjective sleepiness. Because the increase in SWS and δ wave power is thought to be a sensitive indicator of the sleep homeostatic process [63,64], it is possible that $FC_{amg-vACC}$ is

Table 4. Correlations between inter-session differences for fMRI data and questionnaire scores, and objective sleep debt Indices.

	Δamygdala activation	$\Delta FC_{amg-vACC}$
ΔSTAI-state	0.02	−0.82***
ΔPOMS Vigor	−0.22	0.49
ΔPOMS Depression	−0.20	−0.46
ΔPOMS Anger-Hostility	−0.38	0.11
ΔPOMS Fatigue	−0.27	−0.28
ΔPOMS Tension-Anxiety	0.13	−0.73**
ΔPOMS Confusion	0.02	−0.60*
ΔSSS	−0.41	0.01
ΔSWS_{2 hrs}	0.36	−0.59*
ΔDelta_{2 hrs}	0.03	−0.55*

Note. Δvalue, inter-session difference between sleep control and sleep debt sessions for each value; $FC_{amg-vACC}$, functional connectivity between amygdala and ventral ACC; SSS, Stanford Sleepiness Scale; STAI, State-Trait Anxiety Inventory; POMS, Profile of Mood States; $SWS_{2 h}$, slow wave sleep of first 2 h from sleep onset; $Delta_{2 h}$, delta wave power of first 2 h from sleep onset;
*$p < 0.05$,
**$p < 0.01$,
***$p < 0.001$.
Degrees of freedom (df) = 13.
doi:10.1371/journal.pone.0056578.t004

associated with the neural basis that enhances homeostatic sleep pressure following short hours of sleep. It is also thought that the increase in homeostatic sleep pressure is caused by the accumulation of sleep promoting substances in brain, such as prostaglandin D2 and adenosine [65,66,67,68,69]. For example, adenosine receptor antagonists have been shown to reduce sleep time by increasing wake hours and reduce slow wave activity during sleep [65,66,68]. Recent studies have hypothesized that adenosine, accumulated in the synaptic cleft because of consecutive synaptic transmission, binds to the A1 receptor to suppress synaptic transmission [70]. It is possible that synaptic suppression by a sleep-promoting substance is involved in the attenuation of $FC_{amg-vACC}$ during sleep debt. Functional connectivity of the BOLD signal reportedly reflects a synaptic anatomical pathway [71]; therefore, it is expected that synaptic suppression by adenosine causes a decline in functional connectivity. This hypothesis is supported by a previous study which reported that a lack of sleep leads to attenuated resting state functional connectivity [72]. Moreover, a positron emission tomography (PET) study of human subjects revealed increased adenosine binding to the A1 receptor in regions including the vACC during sleep deprivation [73]. The use of PET with fMRI to elucidate the effects of sleep-promoting substances such as adenosine, including the effect of adenosine on the dynamics of neural activity in the brain, will likely elucidate the neural basis that alters the mechanism of emotion regulation.

Some limitations need to be taken into account when interpreting the present findings. First, participants in this study spent the first 3 days at home. Even though their sleep schedule was enforced by the use of the actigraph and mail alerts, their actual sleep time (8 h 5 min and 4 h 36 min in the SC and SD session, respectively) was longer than the scheduled sleep time (8 and 4 h in the SC and SD session, respectively). Despite the minor increase in sleep time, the SD session had a sleep loss of 3 h 30 min compared with the SC session. This level of sleep loss is rather frequently experienced in everyday life; however, the presence of sleep debt was confirmed on the last day of the session

by the increase in %SWS and δ wave power. Because sleep requirements vary among individuals, the same sleep schedule may result in large individual differences in sleep debt [74]. In other words, even if everyone were to be placed strictly on the same sleep schedule, the effects of sleep debt on brain activity and subjective moods would vary widely among individuals. If sleep can be restricted based on individual sleep requirements, functional changes in the amygdala and $FC_{amg\text{-}vACC}$ might be identified more accurately.

Conclusion

The results of this study indicate that a short-term sleep loss, which is often experienced in everyday life, can aggravate subjective mood including anxiety, and the mechanism appears to involve functional alteration of the amygdala and $FC_{amg\text{-}vACC}$. Long work hours, night-owl lifestyles, and an increase in shift work are the major contributors to sleep loss and thus the risk for

depression [1,2,4,6,75,76,77,78]. Therefore, ensuring adequate sleep is an important lifestyle factor that deserves more attention in terms of managing mental health, including depression.

Acknowledgments

Author Contributions

Conceived and designed the experiments: Y. Motomura SK KO YT Y. Moriguchi KM. Performed the experiments: Y. Motomura SK KO YT ME YK AH. Analyzed the data: Y. Motomura SK ME. Contributed reagents/materials/analysis tools: Y. Motomura SK KO YT ME Y. Moriguchi. Wrote the paper: Y. Motomura SK Y. Moriguchi SH KM.

References

1. Basner M, Fomberstein KM, Razavi FM, Banks S, William JH, et al. (2007) American time use survey: sleep time and its relationship to waking activities. Sleep 30: 1085–1095.
2. Bonnet MH, Arand DL (1995) We are chronically sleep deprived. Sleep 18: 908–911.
3. Breslau N, Roth T, Rosenthal L, Andreski P (1997) Daytime sleepiness: an epidemiological study of young adults. Am J Public Health 87: 1649–1653.
4. Hublin C, Kaprio J, Partinen M, Koskenvuo M (2001) Insufficient sleep–a population-based study in adults. Sleep 24: 392–400.
5. Kaneita Y, Ohida T, Uchiyama M, Takemura S, Kawahara K, et al. (2005) Excessive daytime sleepiness among the Japanese general population. J Epidemiol 15: 1–8.
6. Rajaratnam SMW, Arendt J (2001) Health in a 24-h society. The Lancet 358: 999–1005.
7. Carskadon MA, Dement WC (1981) Cumulative effects of sleep restriction on daytime sleepiness. Psychophysiology 18: 107–113.
8. Chuah LY, Chee MW (2008) Functional neuroimaging of sleep deprived healthy volunteers and persons with sleep disorders: a brief review. Ann Acad Med Singapore 37: 689–694.
9. Durmer JS, Dinges DF (2005) Neurocognitive consequences of sleep deprivation. Seminars in Neurology 25: 117–129.
10. Franzen PL, Buysse DJ, Dahl RE, Thompson W, Siegle GJ (2009) Sleep deprivation alters pupillary reactivity to emotional stimuli in healthy young adults. Biol Psychol 80: 300–305.
11. Zhong X, Hilton HJ, Gates GJ, Jelic S, Stern Y, et al. (2005) Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. J Appl Physiol 98: 2024–2032.
12. Chuah LY, Dolcos F, Chen AK, Zheng H, Parimal S, et al. (2010) Sleep deprivation and interference by emotional distracters. Sleep 33: 1305–1313.
13. Minkel JD, Banks S, Htaik O, Moreta MC, Jones CW, et al. (2012) Sleep Deprivation and Stressors: Evidence for Elevated Negative Affect in Response to Mild Stressors When Sleep Deprived. Emotion 12: 1015–1020.
14. Yoo S-S, Gujar N, Hu P, Jolesz FA, Walker MP (2007) The human emotional brain without sleep — a prefrontal amygdala disconnect. Current Biology 17: R877–R878.
15. Swann CE, Yelland GW, Redman JR, Rajaratnam SM (2006) Chronic partial sleep loss increases the facilitatory role of a masked prime in a word recognition task. J Sleep Res 15: 23–29.
16. Killgore WD, Yurgelun-Todd DA (2004) Activation of the amygdala and anterior cingulate during nonconscious processing of sad versus happy faces. Neuroimage 21: 1215–1223.
17. Morris JS, Ohman A, Dolan RJ (1999) A subcortical pathway to the right amygdala mediating "unseen" fear. Proc Natl Acad Sci U S A 96: 1680–1685.
18. Nomura M, Ohira H, Haneda K, Iidaka T, Sadato N, et al. (2004) Functional association of the amygdala and ventral prefrontal cortex during cognitive evaluation of facial expressions primed by masked angry faces: an event-related fMRI study. Neuroimage 21: 352–363.
19. Pessoa L, Adolphs R (2010) Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. Nat Rev Neurosci 11: 773–783.
20. Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, et al. (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 18: 411–418.
21. Etkin A, Klemenhagen KC, Dudman JT, Rogan MT, Hen R, et al. (2004) Individual Differences in Trait Anxiety Predict the Response of the Basolateral Amygdala to Unconsciously Processed Fearful Faces. Neuron 44: 1043–1055.
22. Williams LM, Das P, Liddell BJ, Kemp AH, Rennie CJ, et al. (2006) Mode of functional connectivity in amygdala pathways dissociates level of awareness for signals of fear. J Neurosci 26: 9264–9271.
23. Banks S, Dinges DF (2007) Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 3: 519–528.
24. Banks S, Van Dongen H, Dinges DF (2010) Effect of Sleep Dose on Recovery Sleep Stage and Slow Wave Energy Dynamics Following Chronic Sleep Restriction. Sleep 33: A104–A104.
25. Belenky G, Wesensten NJ, Thorne DR, Thomas ML, Sing HC, et al. (2003) Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study. Journal of Sleep Research 12: 1–12.
26. Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, et al. (1997) Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4–5 hours per night. Sleep 20: 267–277.
27. Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26: 117–126.
28. Zohar D, Tzischinsky O, Epstein R, Lavie P (2005) The effects of sleep loss on medical residents' emotional reactions to work events: a cognitive-energy model. Sleep 28: 47–54.
29. Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC (1992) Automatic sleep/wake identification from wrist activity. Sleep 15: 461–469.
30. Ekman P, Friesen WV (1971) Constants across cultures in the face and emotion. J Pers Soc Psychol 17: 124–129.
31. Ogawa T, Oda M, Yoshikawa S, Akamatsu S (1997) Evaluation of Facial Expressions Differing in Face Angles: Constructing a Database of Facial Expressions. The Institute of Electronics, Information and Communication Engineers, technical report, HIP, Human Information Processing 97: 47–52.
32. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC (1973) Quantification of sleepiness: a new approach. Psychophysiology 10: 431–436.
33. Spielberger CD, Gorsuch RL (1970) STAI Manual for the Stait-Trait Anxiety Inventory.
34. McNair DM, Lorr M, Droppleman LF (1971) Profile of Mood States (POMS™).
35. Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects(National Institutes of Health).
36. Agnew HW, Jr., Webb WB, Williams RL (1966) The first night effect: an EEG study of sleep. Psychophysiology 2: 263–266.
37. Akerstedt T, Kecklund G, Ingre M, Lekander M, Axelsson J (2009) Sleep homeostasis during repeated sleep restriction and recovery: support from EEG dynamics. Sleep 32: 217–222.
38. Borbely AA, Achermann P (1999) Sleep homeostasis and models of sleep regulation. J Biol Rhythms 14: 557–568.
39. Brunner DP, Dijk DJ, Borbely AA (1993) Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness. Sleep 16: 100–113.
40. Dijk DJ, Hayes B, Czeisler CA (1993) Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. Brain Res 626: 190–199.
41. Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, et al. (1996) A unified statistical approach for determining significant signals in images of cerebral activation. Hum Brain Mapp 4: 58–73.
42. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, et al. (1997) Psychophysiological and modulatory interactions in neuroimaging. Neuroimage 6: 218–229.

43. Gloor P, Olivier A, Quesney LF, Andermann F, Horowitz S (1982) The role of the limbic system in experiential phenomena of temporal lobe epilepsy. Ann Neurol 12: 129–144.

44. Halgren E, Walter RD, Cherlow DG, Crandall PH (1978) Mental phenomena evoked by electrical stimulation of the human hippocampal formation and amygdala. Brain 101: 83–117.

45. Phelps EA (2006) Emotion and cognition: insights from studies of the human amygdala. Annu Rev Psychol 57: 27–53.

46. Fitzgerald DA, Angstadt M, Jelsone LM, Nathan PJ, Phan KL (2006) Beyond threat: amygdala reactivity across multiple expressions of facial affect. Neuroimage 30: 1441–1448.

47. Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, et al. (2009) Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. Journal of Psychiatry & Neuroscience 34: 418–432.

48. Dannlowski U, Ohrmann P, Bauer J, Kugel H, Baune BT, et al. (2007) Serotonergic genes modulate amygdala activity in major depression. Genes Brain Behav 6: 672–676.

49. McClure EB, Monk CS, Nelson EE, Parrish JM, Adler A, et al. (2007) Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. Arch Gen Psychiatry 64: 97–106.

50. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, et al. (2001) Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: An fMRI study. Biological Psychiatry 50: 651–658.

51. Kim MJ, Loucks RA, Palmer AL, Brown AC, Solomon KM, et al. (2011) The structural and functional connectivity of the amygdala: From normal emotion to pathological anxiety. Behavioural Brain Research 223: 403–410.

52. Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007) Amygdala-frontal connectivity during emotion regulation. Soc Cogn Affect Neurosci 2: 303–312.

53. Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, et al. (2006) Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. J Neurosci 26: 4415–4425.

54. Hampton AN, Adolphs R, Tyszka MJ, O'Doherty JP (2007) Contributions of the amygdala to reward expectancy and choice signals in human prefrontal cortex. Neuron 55: 545–555.

55. Murray EA (2007) The amygdala, reward and emotion. Trends Cogn Sci 11: 489–497.

56. Gujar N, Yoo SS, Hu P, Walker MP (2011) Sleep deprivation amplifies reactivity of brain reward networks, biasing the appraisal of positive emotional experiences. J Neurosci 31: 4466–4474.

57. Bliss EL, Clark LD, West CD (1959) Studies of sleep deprivation-relationship to schizophrenia. AMA Arch Neurol Psychiatry 81: 348–359.

58. Dahl RE (2004) Regulation of sleep and arousal: comments on part VII. Ann N Y Acad Sci 1021: 292–293.

59. Gillin JC, Buchsbaum M, Wu J, Clark C, Bunney W, Jr. (2001) Sleep deprivation as a model experimental antidepressant treatment: findings from functional brain imaging. Depress Anxiety 14: 37–49.

60. Horne JA (1993) Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. Br J Psychiatry 162: 413–419.

61. Wirz-Justice A, Van den Hoofdakker RH (1999) Sleep deprivation in depression: what do we know, where do we go? Biol Psychiatry 46: 445–453.

62. Norbury R, Taylor MJ, Selvaraj S, Murphy SE, Harmer CJ, et al. (2009) Short-term antidepressant treatment modulates amygdala response to happy faces. Psychopharmacology (Berl) 206: 197–204.

63. Achermann P, Dijk DJ, Brunner DP, Borbely AA (1993) A model of human sleep homeostasis based on EEG slow-wave activity: quantitative comparison of data and simulations. Brain Res Bull 31: 97–113.

64. Borbely AA, Baumann F, Brandeis D, Strauch I, Lehmann D (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51: 483–495.

65. Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Prog Neurobiol 73: 379–396.

66. Benington JH, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45: 347–360.

67. Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, et al. (2008) Sleep as a fundamental property of neuronal assemblies. Nat Rev Neurosci 9: 910–919.

68. Landolt HP (2008) Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol 75: 2070–2079.

69. Urade Y, Hayaishi O (2011) Prostaglandin D2 and sleep/wake regulation. Sleep Med Rev 15: 411–418.

70. Jones BE (2009) Glia, adenosine, and sleep. Neuron 61: 156–157.

71. Lu J, Liu H, Zhang M, Wang D, Cao Y, et al. (2011) Focal pontine lesions provide evidence that intrinsic functional connectivity reflects polysynaptic anatomical pathways. J Neurosci 31: 15065–15071.

72. Killgore WDS, Schwab ZJ, Weiner MR (2012) Self-reported nocturnal sleep duration is associated with next-day resting state functional connectivity. Neuroreport 23: 741–745.

73. Elmenhorst D, Meyer PT, Winz OH, Matusch A, Ermert J, et al. (2007) Sleep deprivation increases A1 adenosine receptor binding in the human brain: a positron emission tomography study. J Neurosci 27: 2410–2415.

74. Van Dongen HP, Baynard MD, Maislin G, Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep 27: 423–433.

75. Chalupka S (2012) Overtime Work as a Predictor of a Major Depressive Episode. Workplace Health & Safety 60: 192–192.

76. Kitamura S, Hida A, Watanabe M, Enomoto M, Aritake-Okada S, et al. (2010) Evening preference is related to the incidence of depressive states independent of sleep-wake conditions. Chronobiol Int 27: 1797–1812.

77. Scott AJ, Monk TH, Brink LL (1997) Shiftwork as a Risk Factor for Depression: A Pilot Study. Int J Occup Environ Health 3: S2–S9.

78. Virtanen M, Stansfeld SA, Fuhrer R, Ferrie JE, Kivimaki M (2012) Overtime Work as a Predictor of Major Depressive Episode: A 5-Year Follow-Up of the Whitehall II Study. Plos One 7.