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Neurophysiological effects of sleep deprivation in healthy adults

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ABSTRACT

Background: Total sleep deprivation (TSD) may induce fatigue, neurocognitive slowing and mood changes, which are partly compensated by stress regulating brain systems, resulting in altered dopamine and cortisol levels in order to stay awake if needed. These systems, however, have never been studied in concert.

Methods: At baseline, after a regular night of sleep, and the next morning after TSD, 12 healthy subjects performed a semantic affective classification task, adapted for use during functional magnetic resonance imaging (fMRI). Saliva cortisol levels were acquired at 7 time points during both days. [¹¹C]Raclopride binding was determined using positron emission tomography (PET). Affective symptoms were measured using Beck Depression Inventory (BDI), Spielberger State Trait Anxiety Index (STAI) and visual analogue scales.

Results: After TSD, perceived energy levels, concentration, and speed of thought decreased significantly, whereas mood did not. During fMRI, response speed decreased for neutral words and positive targets, and accuracy decreased trendwise for neutral words and for positive targets with a negative distracter. Following TSD, processing of positive words was associated with increased bilateral prefrontal activation, whereas negative words activated left insula and right dorsolateral prefrontal cortex. Cortisol secretion was significantly lower after TSD. Decreased [¹¹C]raclopride binding was observed in left caudate, putamen and thalamus.

Conclusions: TSD induces widespread cognitive, neurophysiologic and endocrine changes in healthy adults, characterized by reduced cognitive functioning, despite increased regional brain activity. The blunted HPA-axis response together with decreased [¹¹C]raclopride binding in the basal ganglia indicate that sustained wakefulness requires involvement of additional adaptive biological systems.

INTRODUCTION

Lack of sleep is a common condition in everyday life, either related to psychosocial demands or related to working shift hours. In healthy individuals, this may induce decreased alertness and vigilance, together with a general decline in mood (1). Total sleep deprivation (TSD) has been associated with general psychomotor slowing and diminished cognitive performance, but is also known to have beneficial effects on mood in depressive disorder (2,3). Thus, affect clearly responds to sleep deprivation.

From an evolutionary perspective, staying awake has served to guard against outside threats, requiring increased alertness. Motivational control over the waking state is necessary and presumed to be modulated by top-down cortical control systems, involving prefrontal executive regions (1). Using [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) as a ligand in positron emission tomography (PET) studies, sleep deprivation has been associated with reduced metabolic activity in a network of brain regions, including prefrontal and limbic regions, the thalamo-basal ganglia circuit, and cerebellum (4,5). Neurophysiologically, dopamine (DA) release is supposed to increase wakefulness, partly through the D2 receptor (6-9) and partly by acting as a stimulator of corticotropin releasing hormone (CRH) (6,10). Ultimately, CRH releases cortisol from the adrenal cortex via the hypothalamic pituitary adrenal (HPA) axis, a key endocrine response mechanism to a stressful situation (11). These effects are superimposed upon the circadian rhythm of the HPA axis, and largely controlled by the central body clock, the suprachiasmatic nucleus (SCN). HPA axis functioning can be assessed by the cortisol awakening response (CAR), reflecting the natural HPA response to stress of sleep-wake transitions (12). It is unknown, however, how cortical, dopaminergic and HPA axis activities interact to maintain wakefulness. Studying their interaction may also provide insight into the pathophysiology of various mood disorders, in particular depressive disorder with its frequently occurring sleeping problems (13) and HPA-axis hyperactivity (11,14,15).

The purpose of this study was to assess how the healthy brain responds to TSD and how compensatory and regulatory stress mechanisms may interact. It was hypothesized that wakefulness would be associated with an increase in dopamine release and CRH activation, in the presence of altered emotional functioning (3).

MATERIALS AND METHODS

Subjects

Twelve healthy adults (6 female, mean age 29.2 ± 10.2 years; 6 male, mean age 28.5 ± 4.8 years) were recruited through newspaper advertisements. Exclusion criteria included a lifetime history of psychiatric disorders, as assessed by Mini international neuropsychiatric interview (16) and reported contacts with mental health counselors, 1st degree relatives with psychiatric disorder, somatic disorders, pregnancy, and past or current abuse of psychoactive drugs known to interfere with the dopaminergic system. All subjects were good sleepers, defined as feeling rested after a night's sleep, and in good physical health as assessed by medical history, physical examination and routine laboratory tests. On the night preceding TSD, subjects slept 6.6 ± 1.1 hours. Mean body mass index was $21.0 \pm 1.4 \text{ kg}\cdot\text{m}^{-2}$, 2 were cigarette smokers (10 per day), and 10 consumed alcohol (1.5 ± 1.1 units per day). Written informed consent was obtained from all participants. The study protocol was approved by the Medical Ethics Review Committee of the VU University Medical Centre in Amsterdam.

Design and Procedure

Cortisol saliva was collected on both days. At baseline, on day 1, all subjects underwent functional magnetic resonance imaging (fMRI) scanning in the morning, followed by a 60 min [^{11}C]raclopride PET scan. After this scanning session, participants returned to their daily activities, including study and/or work. They returned to the hospital at 22.00 h, where urine toxicology was screened and found negative for use of cocaine, tetrahydrocannabinol (THC) and amphetamines. During the night, and at the start of day 2, subjects were monitored by a trained observer and engaged in reading, conversation, short walks on the ward, and board games in a well-lit room. Use of alcohol, caffeine and smoking was prohibited. A light meal was served at 6.00 h. Next, after having been awake for about 25 hours, fMRI scanning was repeated. Finally, after having been awake for about 28 hours, the [^{11}C]raclopride PET was repeated. After finishing the scan sessions, subjects were asked to stay awake during the remainder of the day, and to postpone sleep until the evening.

Psychometric data

Depressive symptoms over the prior week were assessed using the Beck Depression Inventory (17). Before scanning, state anxiety was measured using the Spielberger State-Trait Anxiety Inventory (STAI) (18). During sleep deprivation, self and observer

based visual analogue scales (VAS) were registered every 3 hours, starting at 24.00 h and finishing at 12.00 h, documenting mood, interest, motor inhibition, speed of thought, self appreciation, energy level and concentration on a scale from 0-100 (19). Psychometric data were analyzed using Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPPS Inc, Chicago, Illinois, USA), using Repeated Measures ANOVA.

Cortisol measurements

Data acquisition

Saliva samples were obtained using Salivettes (Starstedt, Germany) at 7 time points per day. One hour cortisol awakening response (CAR) measurements included three time points, immediately after awakening (T1), at +30 min (T2) and at +60 min (T3). Additional saliva samples were taken at +90 min (T4) after awakening, at 14.00 h (T5), 17.00 h (T6) and 23.00 h (T7). Subjects were instructed to write down the exact sampling time. On the following day, samples were collected at identical time points (T8-T14). Eating, smoking, drinking tea or coffee or brushing teeth was prohibited within 15 min before sampling. No dental work was allowed within 24 hours prior to sampling. Samples were stored in a refrigerator and returned by the participant or by regular mail. Salivettes were centrifuged at 2000 g for 10 min, aliquoted and stored at -80°C. Free cortisol analysis was performed by competitive electrochemiluminescence immunoassay (Architect, Abbott Laboratories, Illinois, USA) (20). The lower limit of quantification was 2.0 nmol·L⁻¹, the intra- and inter-assay variability coefficients were less than 9 and 11%.

Data analysis

The CAR area under the curve (AUC), with respect to increase (AUC_I) and to ground (AUC_G) were calculated. AUC_I is calculated with reference to the baseline measurement at T1, ignoring the distance from zero for all measurements, and emphasizing change over time. AUC_G is the total area under the curve of all measurements (21). The mean increase in the 1st hour (MnInc) was calculated by subtracting the baseline value at T1 from the mean of the subsequent values at T2 and T3. Using the real sampling time at T2, T3, T9 and T10, cortisol levels were interpolated using piecewise linear spline to +30 and +60 min, in order to derive the individual CAR AUC for identical time points on both days (22). For AUC_G T1-T7 and T8-T14, mixed model analysis was used to include time points available, with missing values being interpolated (23).

fMRI

Task design

We used a semantic emotional classification task adapted from Murphy et al. (24) and Elliot et al. (25), based on classic 'go/no-go' methodology, where subjects had to respond as quickly as possible to target stimuli and ignore distracter stimuli (25-27). It comprised a blocked design with 16 active blocks, programmed in E-prime software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Targets and distracters were defined on the basis of emotional valence, with happy, sad, or neutral words as targets, presented with one of the other categories as distracters (e.g. happy targets with sad distracters). All the words were selected from the Centre for Lexical Information (Celex) Database (28), and matched for frequency of written use and word length. Affective words were selected on high emotional impact (positive words 6.0 ± 1.6 letters, intensity 2.2 ± 0.5 ; negative words 5.7 ± 0.4 letters, intensity 5.9 ± 1). A neutral condition was included, where targets and distracters were defined on the basis of physical properties (italic vs. plain font). Eight different task conditions were presented pseudo-randomized over the two scanning sessions, which followed a randomized cross-over design. In each block, 11 targets and 11 distracters were presented in a randomized order. Each block started with a 2 s rest block, followed by a 5 s block with a written instruction, followed by a 1 s rest block. Following a fixation cross for 800 ms, a word was shown for a maximum of 500 ms to which subjects were asked to respond by pressing a button with the preferred index finger, within an additional 900 ms. After pressing, the word was no longer visible. At the end of a block, a 1 s rest was included prior to the next block. After two practice blocks, 14 active blocks were presented.

Data acquisition

T1-weighted MRI scans were acquired using a 1.5T Sonata MR system (Siemens Medical Solutions, Erlangen, Germany) to exclude anatomical abnormalities and for PET and fMRI co-registration purposes. A sagittal 3D gradient-echo T1-weighted image was acquired using the following sequence: repetition time (TR) = 2.7 ms, echo time (TE) = 3.97 ms, matrix 256×160 , voxel size $1 \times 1 \times 1.5 \text{ mm}^3$. Echo-planar images (EPI) were obtained using a T2*-weighted gradient echo sequence TR = 2.18 s, TE = 45 ms, 35 axial slices; voxel size $3 \times 3 \times 3 \text{ mm}^3$, flip angle 90° , matrix 64×64). For the fMRI task, stimuli were projected onto a screen at the end of the scanner table, visible through a mirror mounted above the subject's head. Two magnetic field compatible response boxes were used to record the subject's responses.

Data processing

Functional imaging data were preprocessed and analyzed using Statistical Parametric Mapping (SPM) software (SPM8, Wellcome Trust Neuroimaging Centre, London, UK), implemented in Matlab 7.1.0 (The MathWorks Inc., Natick, MA, USA). Preprocessing included reorientation of the functional images to the anterior commissure, slice time correction, image realignment, co-registration of the T1 scan to the mean image, warping of the co-registered T1 image to Montreal Neurological Institute (MNI) space as defined by SPM's T1 template, applying the transformations to the slice-timed and realigned images, reslicing to voxels of $3 \times 3 \times 3 \text{ mm}^3$ and applying spatial smoothing using an 8 mm full width at half maximum (FWHM) Gaussian kernel. Subject movements of more than 3 mm in more than one direction resulted in exclusion of data.

Data analysis

Contrast images for target-distracter pairs including neutral (*italic vs. plain font*) and emotional (positive and negative valence words vs. neutral or opposite valence words) were first computed at a single-subject level, and subsequently entered into a second level paired-samples *t*-test (day 1 vs. day 2). The main effect of time (day 1 vs. day 2) is reported at a threshold of *p* uncorrected <0.005 , with an extent threshold of 10 contiguous voxels, to balance the risk for Type I and II error, as proposed by Lieberman et al. (29). Psychometric and performance data (correct responses, false alarms, misses and mean response time for events (RT)) for both days were likewise analysed using paired sample *t*-testing.

[¹¹C]Raclopride PET**Data acquisition**

[¹¹C]Raclopride scans were performed on an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Participants were studied at rest, in supine position, with a nurse nearby and ice cubes in both hands to prevent them from falling asleep. Head movement was restricted by a head immobilization device and Velcro tape. A venous catheter was placed in the forearm for [¹¹C]raclopride infusion. A 10 min 2D transmission scan using three rotating ⁶⁸Ge/⁶⁸Ga sources was acquired for photon attenuation correction. 370 MBq [¹¹C]raclopride was dissolved in 5 mL saline and administered by an infusion pump (Med-Rad, Beek, The Netherlands), at a rate of $0.8 \text{ mL}\cdot\text{s}^{-1}$, followed by a 35 mL saline flush at a rate of $2.0 \text{ mL}\cdot\text{s}^{-1}$. Meanwhile, a 60 min dynamic 3D raclopride scan was acquired, consisting of 20 frames with progressively increasing frame lengths (1 x 15, 3 x 5, 3 x 10, 2 x 30, 3 x 60, 2 x 150, 2 x 300, 4 x 600 s).

All PET sinograms were normalized and corrections were applied for decay, dead time, attenuation scatter and randoms. Emission data were reconstructed using FORE+2D filtered back projection (30,31) applying a 5.0 mm Hanning filter with a Y-offset of 4 cm and a 2.123 zoom. Frames 12-20 were summed (i.e. 5-60 min after injection) to create a single frame emission sinogram with high count statistics. Reconstruction of this emission sinogram was performed using ordered-subset expectation maximization (OSEM) with 4 iterations and 16 subsets. OSEM images underwent a 5 mm FWHM Gaussian post smoothing, to obtain a transaxial spatial resolution of 7 mm FWHM, equal to that of filtered back projected (FBP) images (32). Final images consisted of 63 planes of 128 x 128 voxels, each 2.4 x 2.4 x 2.4 mm³.

Data processing

All structural MRI scans were rotated to the axial (horizontal) plane, parallel to the anterior and posterior commissure (AC–PC) line. To correct for possible motion, each frame (1-20) was coregistered to the summed image over frames 12-20. These motion corrected PET images were subsequently coregistered to the realigned MRI scan using Volume Imaging in Neurological Research (VINCI) software (33).

Kinetic analysis

Mean nondisplaceable binding potential (BP_{ND}) was used as a measure of dopamine D2/D3 receptor availability. Using the in-house developed software package PPET (34), parametric BP_{ND} images were generated using receptor parametric imaging (RPM2), a basis function implementation of the simplified reference tissue model (SRTM) (35,36). Cerebellum grey matter was used as reference tissue, for which automated cerebellar volumes of interest (VOIs) were defined using partial volume effect (PVE) lab (37). This analysis also provided parametric R₁ images, representing local tracer delivery relative to that to the reference region. Basis function settings used were: start exponential = 0.05 min⁻¹, end = 0.5 min⁻¹, number of basis functions 32.

Statistical parametric mapping

Parametric BP_{ND} images were analyzed using SPM8. After spatial preprocessing, including reorientation and normalization to MNI space, images were analyzed on a voxel by voxel basis, using a basal ganglia mask created with WFU Pickatlas software (38). No proportional scaling was applied. SPM RPM2 and R₁ BP_{ND} images were entered in paired sample *t*-tests. The threshold was set at *p* uncorrected ≤ 0.005 with an extent threshold of 10 voxels.

RESULTS

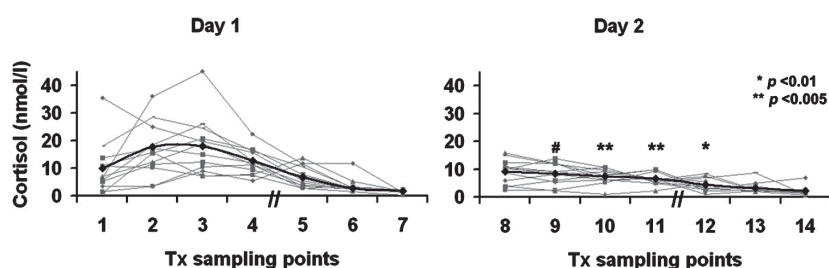
Psychometric data

At baseline, depressive symptoms were low to absent (BDI score 1.8 ± 2.0). During the night, VAS energy levels declined significantly ($F(1,11) = 20.2, p = 0.001$), in line with decreased concentration ($F(1,11) = 10.6, p = 0.01$), speed of thought ($F(1,11) = 12.0, p = 0.007$), and increased perceived motor retardation ($F(1,11) = 12.0, p = 0.007$), but not significantly for mood ($F(1,11) = 2.9, p = 0.122$). STAI scores indicated a trendwise increased anxiousness after TSD ($p = 0.068$).

Cortisol data

After TSD, CAR AUC_i and AUC_G showed significant blunting ($p = 0.029$ and $p = 0.022$, respectively) (Table 1, Figure 1). On day 1, nine subjects showed a rise in cortisol during the first hour after awakening, compared with a much smaller increase in five subjects after TSD, signified by a decreasing MnInc CAR (Table 1). Similarly, cortisol AUC_G T1-7 vs. T8-14 showed a robust decline after TSD. Cortisol levels were normally distributed on both days and showed no significant gender differences. Evening cortisol was not discriminating.

Figure 1



Effects of total sleep deprivation on saliva cortisol levels. Individual saliva cortisol curves (grey line) and cortisol mean value (nmol/L) per Tx sampling point (solid line). Day 1 shows baseline cortisol sampling at T1-T7, day 2 shows effects of one night of total sleep deprivation on cortisol levels at T8-T14. T1, 2 and 3 comprise the cortisol awakening response (CAR). T8, 9 and 10 are sampled at identical time points the following day. T5 and T12 are sampled at 14.00 hr, T6 and T13 at 17.00 hr and T7 and T14 at 23.00 hr. p values show effects of TSD, # $p = 0.016$.

Table 1: Saliva cortisol summary indicators

Day	Indicator	Mean ± Sd	Min	Max	Day	Indicator	Mean ± Sd	Min	Max	p
1	CAR AUC _i	292.08 ± 443.44	-520.35	1290.15	2	CAR AUC _i	-40.65 ± 105.65	-170.25	126.00	0.029
	CAR AUC _i / hr	4.87 ± 7.39	-8.67	21.50		CAR AUC _i / hr	-0.68 ± 1.76	-2.84	2.10	
	CAR AUC _G	886.58 ± 472.21	233.10	1677.15		CAR AUC _G	511.50 ± 208.95	127.95	798.45	0.022
	CAR AUC _G / hr	14.78 ± 7.87	3.89	27.98		CAR AUC _G / hr	8.53 ± 3.48	2.13	13.31	
	MnInc CAR	6.72 ± 10.51	-12.16	30.30		MnInc CAR	-1.05 ± 2.48	-4.26	3.16	0.030
	AUC _G T1-7	6286 ± 2129	2824	11026		AUC _G T8-14	4323 ± 783	2857	6085	0.003
	AUC _G T1-7 / hr	6.55 ± 2.22	2.94	11.49		AUC _G T8-14 / hr	4.50 ± 0.82	2.98	6.34	

n = 162/168 (94.5%) cortisol saliva samples. Units are nmol/l. *p* = paired samples *t*-test for cortisol summary indicators day 1 versus day 2. AUC_i area under the curve, relative to increase, units nmol//hr; AUC_G area under the curve, relative to zero, units nmol//min, or nmol//hr; CAR, cortisol awakening response, including samples at awakening (T1), +30 (T2) and +60 (T3) minutes after awakening; MnInc, Mean Increase in 1st hour after awakening = ((sample T2 + T3)/(2)) - T1.

fMRI

Twelve data sets were available on day 1, and 11 on day 2 due to scanner logistic problems. After TSD, subjects were significantly slower in reacting to the neutral condition ($p = 0.043$), but also to positive targets with a neutral distracter ($p = 0.008$). The proportion of correct versus false answers decreased trendwise for neutral words ($p = 0.082$) and for positive targets with a negative distracter ($p = 0.079$) (Table 2).

After 25 hours of wakefulness, the neutral condition showed no significant activation differences at a group level (Table 3). Evaluation and processing of positive words was associated with increased bilateral prefrontal activation in addition to increased activation of left and middle prefrontal working memory areas (Figure 2A). Processing of negative words resulted in increased activity in left insular area and right dorsolateral prefrontal cortex (DLPFC). During conditions containing emotional words only, viz. positive targets and negative distracters, or vice versa, left insular, limbic and parahippocampal lobes were activated, as well as right parietal lobe (Figure 2B). All emotional conditions (i.e. target and/or distracter) resulted in increased activation in the anterior part of the left insula, mainly driven by the response to words with a negative valence, in addition to activation of the parietal lobe.

[¹¹C]Raclopride

A subset of 8 paired data sets was available due to a failed synthesis (1 TSD scan) and technical problems with 1 baseline and 2 TSD scans. For $n = 8$, injected masses of raclopride were 2.36 ± 1.08 and 1.45 ± 0.55 μg , on days 1 and 2 respectively ($p = 0.06$) and injected doses of [¹¹C]raclopride were 378 ± 12 and 390 ± 19 MBq on days 1 and 2, respectively ($p = 0.230$). TSD resulted in a significantly decreased BP_{ND} in left caudate nucleus, thalamus, and to a smaller extent in putamen, as shown in Table 3 and Figures 2C and 2D. In addition, there was a TSD induced decrease in R_1 in right caudate nucleus.

Table 2: fMRI task results

Target	Condition Distracter	Reaction time (msec) for correct answers			Proportion correct-false answers		
		Day 1		Day 2	Day 1		Day 2
		Mean \pm SD	Mean \pm SD	p value	Mean \pm SD	Mean \pm SD	p value
italics plain	plain italics	460 \pm 98	504 \pm 96	0.043*	0.93 \pm 0.05	0.87 \pm 0.10	0.082†
positive	negative	665 \pm 198	686 \pm 149	0.541	0.92 \pm 0.06	0.87 \pm 0.09	0.079†
negative	positive	673 \pm 173	674 \pm 134	0.977	0.91 \pm 0.07	0.88 \pm 0.10	0.376
neutral	positive	713 \pm 208	754 \pm 181	0.449	0.84 \pm 0.15	0.80 \pm 0.13	0.578
positive	neutral	635 \pm 144	713 \pm 174	0.008**	0.77 \pm 0.12	0.75 \pm 0.17	0.609
neutral	negative	730 \pm 176	760 \pm 176	0.215	0.77 \pm 0.23	0.68 \pm 0.26	0.306
negative	neutral	695 \pm 172	701 \pm 164	0.783	0.80 \pm 0.13	0.76 \pm 0.17	0.606

$n = 11$ pairs, $p =$ paired samples t -test, two-sided

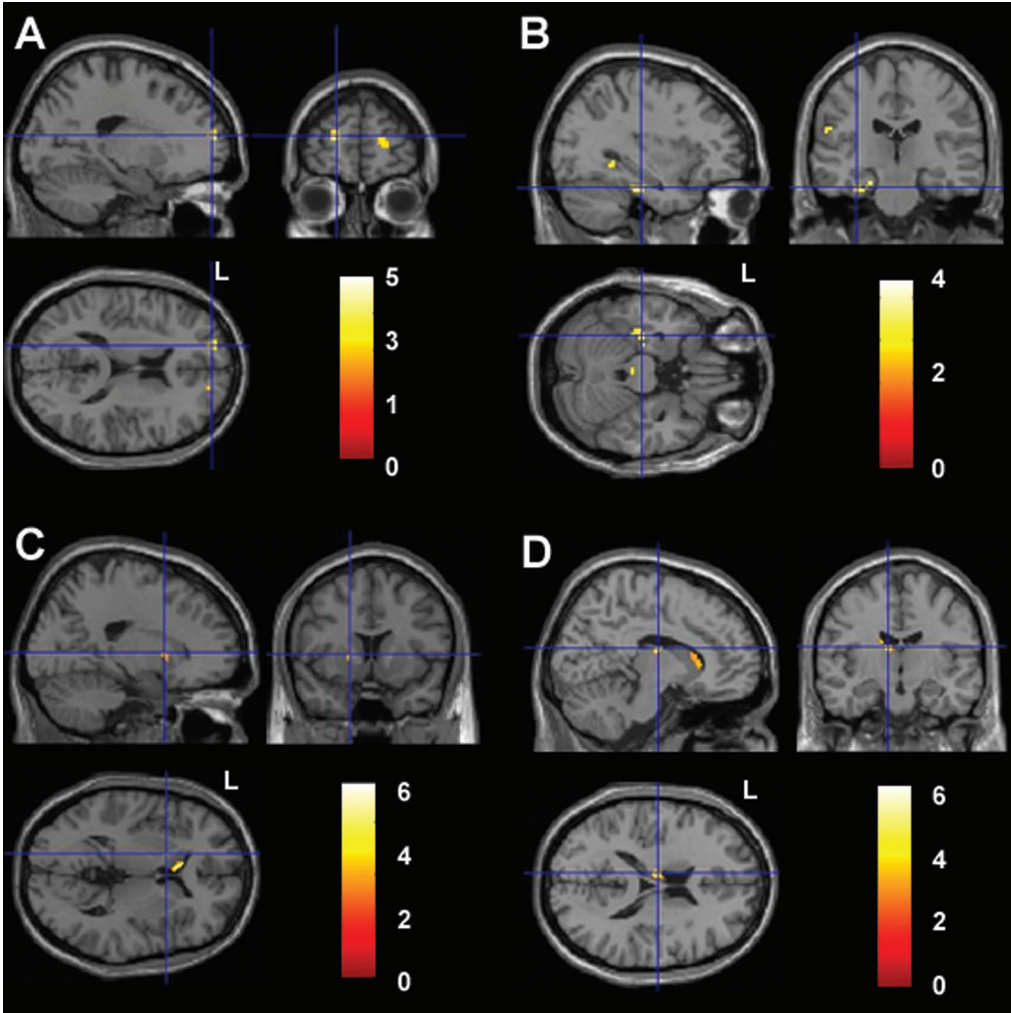
† = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$

Table 3: Neuroimaging effects of TSD for fMRI and PET

	K_E	Z-score	p uncorr	L/R	Region	x	y	z
fMRI, condition TSD > baseline								
Positive valence (target or distracter) vs neutral valence	15	4.03	0.000	L	Superior frontal gyrus	-18	57	15
	10	3.36	0.000	L	Middle frontal gyrus	-30	42	21
	11	3.35	0.000	L	Medial frontal gyrus/ DLPFC	-12	9	45
	12	3.04	0.001	R	Superior frontal gyrus	21	54	9
Negative valence (target or distracter) vs neutral valence	10	3.71	0.000	L	Insula	-42	0	15
	14	3.05	0.001	R	Medial frontal gyrus/ DLPFC	15	57	12
All emotional valences	11	3.66	0.000	R	Parietal lobe	21	-42	39
Positive target/ negative distracter or negative target/ positive distracter	36	3.55	0.000	L	Parahippocampal gyrus	-24	-36	-6
	19	3.41	0.000	L	Parahippocampal gyrus	-33	-24	-24
	10	3.18	0.001	L	Precentral gyrus/ DLPFC	-54	-15	24
	11	3.18	0.001	L	Anterior insula	-27	-9	18
Any emotional target and/ or distracter	17	3.62	0.000	L	Anterior insula	-39	-3	12
	13	3.54	0.000	R	Parietal lobe	21	-42	42
Neutral target and distracter	-	-	-	-	-	-	-	-
[¹¹ C]Raclopride PET, condition TSD < baseline								
RPM2 BP _{ND}	56	3.82	0.000	L	Nucleus caudatus	-8	22	4
	11	2.88	0.002	L	Putamen	-20	10	4
	43	3.79	0.000	L	Thalamus	-12	-12	18
R ₁	88	3.93	0.000	R	Nucleus caudatus	14	0	20

$\rho < 0.005$, extent threshold ten voxels. BP_{ND}, Binding potential; DLPFC, dorsolateral prefrontal cortex; K_E , number of voxels in cluster; L, left; R, right; R₁, relative delivery; RPM, receptor parametric mapping; TSD, total sleep deprivation.

Figure 2



Effects of TSD for fMRI and [^{11}C]raclopride PET at $p < 0.005$, extent threshold 10 voxels. A and B are task related fMRI results, showing increased prefrontal and limbic activation respectively, in the conditions (A) positive valence versus neutral valence and (B) all emotional valences. C and D are [^{11}C]raclopride PET images, showing decreased RPM2 BP_{ND} binding in (C) putamen (sagittal and coronal plane) and nucleus caudatus (transverse plane) and (D) in thalamus (all three planes) and nucleus caudatus (sagittal and coronal plane) in $n = 8$. At the bottom right is the Z-score scale depicted.

DISCUSSION

In the present study the effects of total sleep deprivation on stress regulating brain systems in healthy subjects were investigated. During a sleep deprived night, VAS scores on energy, concentration and speed of thought, but not mood, declined significantly.

After 24 hours of prolonged wakefulness, significant blunting of the cortisol awakening response (CAR) and secretion over the day (AUC_G) were found. Normally, under the influence of the SCN, HPA activity increases during the night, resulting in a cortisol rise two to three hours after sleep onset, which continues to rise into the early waking hours (12,15). The present results indicate robust attenuation of the HPA-mediated stress response after TSD, congruous with decreasing VAS scores and lowered arousal, which may be due to the absence of the initial physiological awakening response (12,39). These findings are in line with Vzontgas and colleagues, finding lowered, albeit not significantly, 24 hour plasma cortisol levels in blood in a laboratory setting in a group of 10 men (40).

In the present study, no significantly altered cortisol levels were found after 14.00 h (T5 and T12). Evening cortisol, indicating return to baseline levels, was slightly lower than those reported by Vreeburg and colleagues (41) in healthy subjects.

After TSD, task performance during fMRI was slower, indicating that TSD overruled any learning or practice effects. Slowing of task performance after TSD is in line with previous reports and likely due to loss of sustained attention and vigilance (42,43). Slowing was particularly evident for positive targets with a neutral distracter. Accuracy was trendwise decreased for the neutral (italic vs. plain font) condition and for positive targets with negative distracters, suggesting decreased sensitivity to detect positive valence. On processing emotionally salient versus neutral words, TSD was associated with increased bilateral involvement of prefrontal areas, suggesting increased mental effort to perform semantic judgements and to maintain control, in a setting of less efficient functional circuitry (44). Processing of solely affective stimuli (target and distracter) showed increased activation in the left parahippocampal gyrus and anterior part of the insula, mainly driven by the response to words with a negative valence, and pointing towards increased effort to handle negative affect (2). These areas are interfaces between emotion and cognition (25), with the insula being specifically related to emotional interference resolution in working memory

(45). Additionally, activation of the insula may be related to performance anxiety as indicated by trendwise increased STAI scores in these healthy, but weary adults (46). The increased activation in the right parietal lobe, as part of the prefrontal-parietal attentional working memory network, underscores the increased effort to maintain top-down control (2,47).

After TSD, BP_{ND} of [^{11}C]raclopride was significantly decreased in left caudate, putamen and thalamus, which is in accordance with a report by Volkow and colleagues (8). This was not explained by altered delivery (R_1) to the basal ganglia. A reduction in [^{11}C]raclopride specific binding is consistent with either an increase in dopamine release, or a decreased affinity of the synaptic D2/D3 receptor in these regions (48). In a recent report, combining clinical and preclinical evidence, Volkow argued decreased [^{11}C]raclopride binding not to be due to increased dopamine availability, but to decreased affinity of the D2/D3 receptor, resulting in dopamine receptor downregulation in the synaptic cleft. As dopamine D2 receptors are thought to be involved in wakefulness, and partially responsible for maintaining arousal and alertness (7,49), the present reduced VAS on energy and concentration and efficiency in fMRI task performance, are in line with D2 downregulation. This would further be exemplified by the blunted cortisol response, since dopaminergic stimulation of the HPA axis is mediated through D1 and D2 receptors (10). Decreased affinity in the head of the left caudate could be in line with increased difficulty in controlling word interference from task unrelated processing (50), explaining both the general slowing and increased prefrontal activity. Decreased affinity in the thalamus may be related to difficulties in maintaining wake state arousal, in a setting of increased sleep pressure (42).

Clinical relevance

Individual vulnerability to sleep deprivation is known to be variable, which is illustrated by the clinical finding that 40-60% of depressed patients benefit from TSD (51), whereas it may induce hypomania in bipolar disorder (52). From the present study, it cannot be ruled out that decreased D2 receptor affinity is the brain's response to initially increased dopamine levels, induced by TSD. Administering the immediate dopamine precursor, L-DOPA, Murphy and co-workers recently reported on inducing a state of hypomania in bipolar disorder (53), whereas no effect on positive mood in healthy volunteers was observed (54), suggesting a mood sensitive dopamine D2 receptor system in bipolar disorder. Blunting of the HPA axis response may reflect the

absence of awakening stress and possibly explain some of the beneficial effects of sleep deprivation in depressive mood disorder.

Limitations

Although the participants' number of hours of sleep of participants was adequate at the start of the experiment, baseline CAR may have been affected by waking up earlier, or by the excitement of taking part in a research study, which may have released additional ACTH (55). A higher CAR has been associated with shorter sleep duration (56), although this has not been a consistent finding (57). During the night, participants were kept in a well-lit room, which may have resulted in melatonin suppression, thereby dampening the SCN mediated CRH response.

Although changes in [^{11}C]raclopride BP_{ND} clearly show a dose dependent relationship with extracellular DA levels, the nature of this relationship is complex (58). Furthermore, [^{11}C]raclopride BP_{ND} represents total ligand receptor binding, and does not differentiate between binding to receptors in high or low affinity states, whereas endogenous dopamine is mainly conveyed by high affinity state receptors (59). [^{11}C]Raclopride only provides information on striatal D2/D3 receptors, whereas dopamine may act on presynaptic and postsynaptic (extra)-striatal dopaminergic D1 receptors to bring about its effect (60). Therefore, dopaminergic effects due to TSD may have been underestimated.

Finally, [^{11}C]raclopride scans were performed in the second half of the morning. As the time sequence of dopamine release is not known, effects may have been either over- or underestimated. A variable response to TSD is in line with observations in depressed patients, where the therapeutic response to TSD may vanish within hours to a day (51).

CONCLUSION

Sleep deprivation in healthy adults induces widespread neurophysiological and endocrine changes, characterized by impaired cognitive functioning, despite increased regional brain activity. Our findings indicate that activation of the dopaminergic system may compensate for a blunted cortisol response, suggesting augmented motivational top down control and requiring increased involvement of prefrontal and limbic

cortical areas. Sustained wakefulness requires the involvement of compensatory brain systems, and may help to understand the therapeutic effects of sleep deprivation in affective disorders.

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