Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature

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FRANKEN, PAUL, DERK-JAN DIJK, IRENE TOBLER, AND ALEXANDER A. BORBÉLY. *Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature.* Am. J. Physiol. 261 (Regulatory Integrative Comp. Physiol. 30): R198–R208, 1991.—Vigilance states, electroencephalogram (EEG) power spectra (0.25–25.0 Hz), and cortical temperature (T_{CRT}) of 10 rats were obtained during a baseline day, a 24-h sleep deprivation (SD) period, and 2 days of recovery (*recoveries 1* and *2*). EEG power density in waking gradually increased in most frequencies during the SD period. Non-rapid-eye-movement (NREM) sleep was enhanced on both recovery days, and rapid-eye-movement sleep was enhanced only on *recovery 1*. In the initial 4 h of *recovery 1*, EEG slow-wave activity (SWA; mean power density 0.75–4.0 Hz) in NREM sleep was elevated relative to baseline, and the number of brief awakenings (*n*BA) was reduced. In the dark period of *recovery 1* and the light period of *recovery 2*, SWA was below baseline, and *n*BA was increased. During the entire recovery period, SWA and *n*BA, both expressed as deviation from baseline values, were negatively correlated. During the SD period, T_{CRT} was above baseline, and in the initial 16 h of *recovery 1* it was below baseline. Whereas T_{CRT} was negatively correlated with NREM sleep, no significant correlation was found between T_{CRT} and SWA within NREM sleep. It is concluded that SD causes a short-lasting intensification of sleep, as indicated by the enhanced SWA and the reduced *n*BA, and a long-lasting increase in sleep duration. The different time courses of SWA and T_{CRT} suggest that variations in NREM sleep intensity are not directly related to changes in T_{CRT}.

electroencephalogram spectral analysis; slow-wave activity; sleep intensity; sleep regulation

IN THE RAT and other rodents, slow-wave activity [SWA; mean electroencephalogram (EEG) power density 0.75–4.0 Hz] exhibits marked variations over the 24-h light-dark (rest-active) cycle. SWA is high at the beginning of the major rest period and declines thereafter (5, 10, 17, 19, 23). Sleep deprivation (SD) by forced locomotion has been used to investigate the processes underlying this variation. After 24 h of SD, SWA is markedly enhanced (4, 17). This is likely to represent a compensatory response to the increased duration of waking. A dose-response relationship between the duration of SD and SWA has been established, and SD as short as 3 h has been shown to elevate SWA (17, 18). The time course of SWA under baseline conditions and its increase after SD have been explained by assuming that this EEG parameter represents an intensity parameter of non-rapid-eye-movement (NREM) sleep and that the underlying process is under homeostatic control (2).

The enhancement of SWA after SD dissipates in the course of recovery sleep, and even a decline below baseline levels has been observed in experiments in which recovery was recorded over a prolonged time span (3, 6, 22). This "negative rebound" is not readily explained in terms of sleep homeostasis and has not yet been investigated by spectral analysis. If SWA indeed reflects the intensity of NREM sleep, it follows that during this negative rebound sleep should be less intense. To test this hypothesis, we analyzed SWA and sleep continuity, which have both been considered indexes of sleep intensity (2, 6), during a 48-h period of recovery from 24 h of SD. A novel aspect of the present experiment was that "gentle handling" instead of forced locomotion was used for SD. This method was applied in an effort to minimize stress during the enforced waking and to exclude locomotion as a confounding variable.

Sleep and temperature regulation are known to be closely related (7, 9, 13, 14, 24, 25). Brain temperature decreases at the onset of NREM sleep and is then regulated at a lower level than in waking. However, it is not known whether SWA within NREM sleep covaries with brain temperature. Because in human studies a short-lasting heat load enhanced slow-wave sleep (SWS; NREM sleep stages 3 and 4) in the subsequent sleep period (8, 15), intensification of recovery sleep in the rat could be due to an increase in body or brain temperature during SD. We therefore analyzed the relationship between SWA and cortical temperature (T_{CRT}) before and after SD.

METHODS AND EXPERIMENTAL PROCEDURES

Animals. Experimental subjects were 10 adult male albino rats of the Sprague-Dawley SIVZ strain with a mean body weight of 357 ± 25 (SE) g. The animals were individually kept in Plexiglas cages (36 × 20 × 35 cm) placed in sound-attenuated chambers. Food and water were available ad libitum. The animals were maintained in a 12:12 h light-dark cycle (light at 0900–2100 h; daylight-type fluorescent tubes, 18 W, ~300 lx). The temperature inside the chambers was 22.9–24.6°C. The animals were adapted to these conditions for 18 days preceding the experiment.

EEG and electromyogram (EMG) electrodes and a thermistor were implanted under deep pentobarbital sodium anesthesia (50 mg/kg ip) 10 days before the experiment. Two gold-plated round-tip miniature screws

(diam 1.19 mm) served as EEG electrodes and were screwed through the skull onto the dura over the right cortex (2.0 mm lateral to the midline, 3.5 mm posterior to the bregma) and the cerebellum (at the midline, 1.5 mm posterior to the lambda). A third screw was placed 1.5 mm left of the midline and 3 mm anterior to the bregma. Two gold wires (diam 0.2 mm) were inserted into the neck muscle tissue to record the muscle activity. A thermistor (Uni-Curve UUB31J1, Fenwal Electronics; resistance at 25°C = 1 kΩ, constant current = 1 mA, maximum diam = 2.0 mm) was used to measure T_{CRT}. The thermistor was inserted between the skull and dura through a hole over the left cortex (2.5 mm lateral to the midline, 2.5 mm posterior to the bregma). The four electrodes and the thermistor were soldered to a connector that was fixed to the skull and anchored to the three screws with dental cement. Three days before the experiment, all animals were connected to counterbalanced recording leads for habituation.

Data acquisition. From each animal nine consecutive 12-h recordings of the EEG, EMG, and T_{CRT} were obtained. The EEG and EMG signals were amplified (amplification factor 2,000, band-pass filter 0.016–200 Hz, −3 dB points, 24 dB/octave) and recorded on paper (Grass polygraph 7D, paper speed 5 mm/s) as well as on analog frequency-modulation magnetic tape (Hewlett-Packard model 3968, tape speed 1.19 cm/s). T_{CRT} and the ambient temperature inside the chambers were recorded for 8-s epochs and stored on hard disk (Olivetti PC M28); simultaneously these epochs were marked on the polygraph paper. Fifteen minutes before the end of each 12-h period, the recordings were interrupted for 15 min to change tapes and the polygraph paper. Each day a calibration signal (10-Hz sine wave, 300 μV peak to peak) was recorded on the EEG channels of the tape and the polygraph paper.

After the experiment the EEG and EMG signals were played back from tape. The EEG signal was low-pass filtered (−3 dB at 30 Hz, 24 dB/octave) and analog-to-digital converted (sampling rate 64 Hz), and a spectral analysis (fast-Fourier transform routine, Digital Equipment Laboratory Subroutine package) was performed on a PDP 11/34 computer as in previous studies (4, 23). EEG power density values were computed for consecutive 4-s epochs in the frequency range from 0.25 to 25.0 Hz (between 0.25 and 5.0 Hz the values were collapsed into 0.5-Hz bins and between 5.25 and 25.0 Hz into 1-Hz bins). The EMG was analog-to-digital converted, full-wave rectified, and integrated over 4-s epochs. To match the T_{CRT} values measured at 8-s intervals with the EEG and EMG values recorded over 4-s epochs, two 4-s values of EEG spectra and EMG were averaged. The vigilance states of these 8-s epochs were determined on the basis of the EEG and EMG signals recorded on the polygraph paper with the help of the EEG power densities in the delta (0.75–4.0 Hz) and the theta bands (6.25–9.0 Hz) and the integrated EMG value, which were displayed on a personal computer monitor. As done previously (23), we discriminated among waking (high EMG amplitude, low EEG amplitude), NREM sleep (low EMG amplitude, high EEG amplitude with high power density in the delta band), and rapid-eye-movement (REM) sleep (low EMG amplitude, low EEG amplitude with high values in the theta band). The vigilance states were entered into the personal computer via the keyboard. Sleep continuity was measured by determining the number of brief awakenings (*n*BA) expressed relative to the amount of total sleep. A brief awakening consisted of a single or two consecutive 8-s epochs scored as waking. Epochs containing EEG artifacts were marked and omitted from further analysis of the power spectra [5.3 ± 0.6% (SE) of total recording time]. The synchronization between spectral data and T_{CRT} was verified by means of a marker on the polygraph paper; the two data sets did not differ by >4 s over a 12-h recording period.

Experimental protocol. The experiment was conducted on 4 consecutive days during which EEG, EMG, and T_{CRT} were recorded continuously. On the 1st day, starting at lights on, baseline recordings were obtained. On the 2nd day animals were deprived of sleep in their home cage. During the 24-h SD period the animals and their polygraph records were continuously observed. When the animals displayed a synchronized high-amplitude EEG pattern or assumed a sleep posture, they were given objects to play with and activated by acoustic and if necessary tactile stimuli. Special care was taken not to interfere with feeding, which sometimes was accompanied by high-amplitude slow waves in the EEG. Recovery was recorded for 2 days (*recoveries 1* and *2*).

Statistical analysis. All 8-s data were averaged over 2-h intervals. The spectral data were expressed relative to an individual reference value (see RESULTS). The spectral data of one animal had to be discarded because of EEG artifacts. Overall effects were analyzed by subjecting the log-transformed values of the relative power densities to analyses of variance (ANOVAs) for each frequency bin. Whenever significant effects were present, paired *t* tests on the log-transformed values were used to further evaluate differences from the reference level.

For clarity of presentation, 6-h geometric means were calculated for the spectral data in waking and NREM sleep during SD (see Fig. 4) and in waking and REM sleep during recovery (see Fig. 5).

The 2-h mean values of T_{CRT} and the vigilance states for the SD and recovery days were compared with the corresponding baseline intervals and analyzed as described above. For T_{CRT} and the vigilance states, 12- and 24-h means were compared with the baseline mean by paired *t* tests. The vigilance states were calculated as percentages of artifact-free recording time. In 1.2 ± 0.5% (SE) of total recording time no vigilance state could be determined because of artifacts.

RESULTS

A continuous 96-h record of T_{CRT}, SWA, and the vigilance states of an animal has been plotted in Fig. 1. In the light period of the baseline day SWA declined. In the dark period no obvious overall trend was present. There was a clear 24-h modulation of T_{CRT}, with low values in the light period and high values in the dark period. Note the increase in T_{CRT} during waking and REM sleep episodes. During SD T_{CRT} was elevated. In the initial hours of *recovery 1*, SWA was higher than the

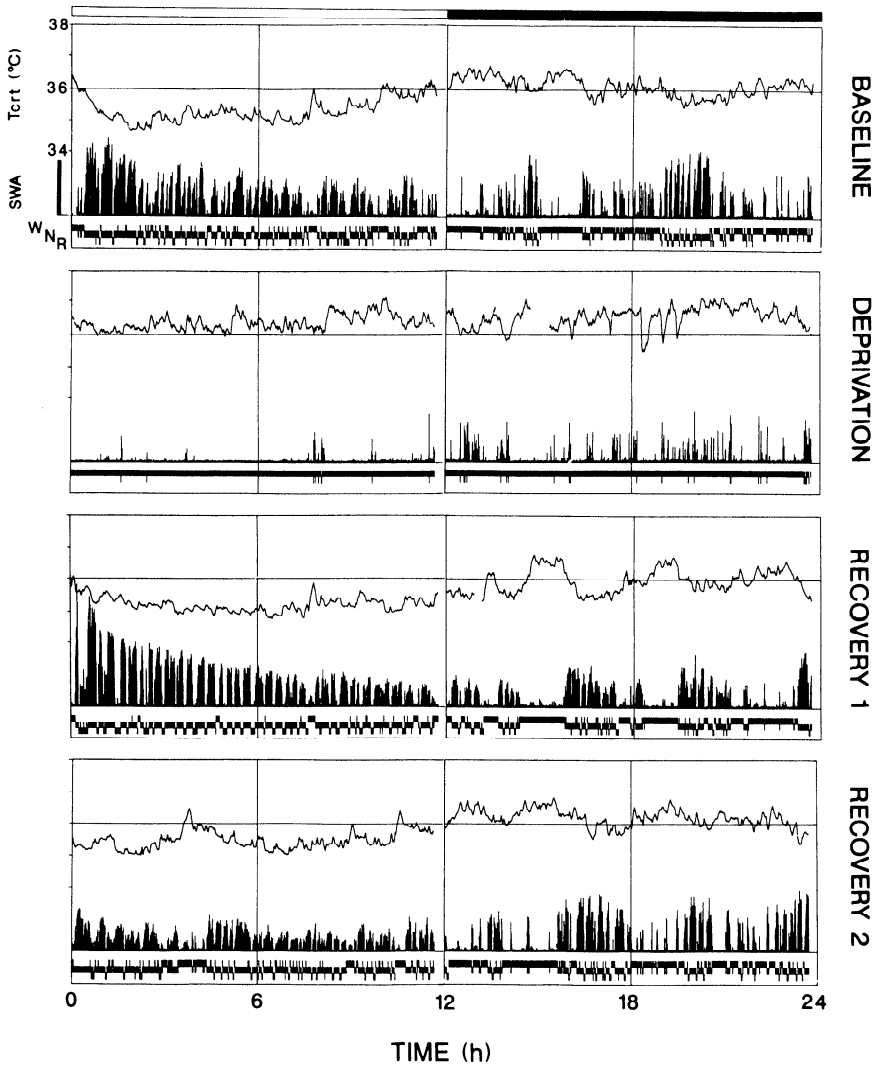

FIG. 1. Cortical temperature (T_{CRT}), electroencephalogram (EEG) slow-wave activity (SWA), and vigilance states [W, waking; N, non-rapid-eye-movement sleep (NREMS); R, rapid-eye-movement sleep (REMS)] of a rat recorded continuously for 4 days. Conditions are indicated at *right*. SWA indicates mean power density in 0.75- to 4.0-Hz band. Data represent mean values over 6 8-s epochs. EEG calibration mark at *top left* corresponds to 1,000 μV^2/Hz.

corresponding baseline level, and the amount of waking was lower. SWA decreased below baseline in the course of the light period. There was an immediate large increase in the amount of REM sleep. During the dark period of *recovery 1* and the light period of *recovery 2*, SWA remained below baseline. In the light period of *recovery 2*, the typical declining trend in SWA was absent.

Vigilance states. To examine the effect of experimental day and time of day on the distribution of the vigilance states, the 2-h values were statistically analyzed by use of a 3 (day: baseline and *recoveries 1* and *2*) by 12 (2-h interval: 1–12) ANOVA with repeated measures on both factors. The factors "day" and "2-h interval" were significant for all three vigilance states ($P < 0.01$). A significant interaction between day and 2-h interval was present for REM sleep and REM sleep per total sleep (TS), which indicates changes in their time course.

The amounts of the vigilance states differed markedly in the light and the dark periods of baseline (Table 1). Within the light and dark periods, the variations of the vigilance states were relatively small, although waking was reduced in the middle of the dark period. The largest changes occurred within the 2 h before and after the light-to-dark or dark-to-light transition.

In the course of the SD it became increasingly difficult to keep the animals awake, and short sleep periods could not be prevented. Episodes with a synchronized high-amplitude EEG and a concomitant low EMG were scored as NREM sleep (Fig. 1). During the light period, the amount of NREM sleep increased progressively from 0.1 to 4.2%. In the dark period it leveled off at ~5%. No REM sleep occurred during the SD day (Table 1).

In *recovery 1*, the 24-h values of all sleep stage parameters were increased relative to baseline, whereas waking was reduced (Table 1, Fig. 2). The REM sleep deficit incurred by the SD was almost fully compensated within 1 day. Especially in the 12-h light period, REM sleep showed a massive increase. NREM sleep showed an increase in the 12-h light period, although a significant rise was present only in one 2-h interval. The REM sleep-to-TS ratio exceeded baseline. The increase in NREM sleep was more prominent in the dark period, whereas the increase in REM sleep was more prominent in the light period. Waking was markedly reduced in both 12-h periods.

In *recovery 2*, the 24-h value as well as both 12-h values of NREM sleep and TS were increased, whereas waking was reduced (Table 1, Fig. 2). As in *recovery 1*, the increase in NREM sleep was more prominent in the dark

TABLE 1. *Vigilance states, total sleep, and percentage of REM sleep per total sleep for 2-h intervals of baseline day and for 12- and 24-h intervals of all experimental days*

Interval No.	NREM Sleep	REM Sleep	TS	REM Sleep/TS	Waking
			2-h Means		
Baseline					
Light					
1	65.8±3.0	7.4±0.9	73.3±3.1	10.2±1.2	26.7±3.1
2	57.8±3.1	13.7±1.2	71.5±3.7	19.1±1.3	28.5±3.7
3	55.1±2.4	13.5±1.5	68.6±3.7	19.2±1.4	31.4±3.7
4	57.1±3.1	13.5±1.4	70.6±3.6	19.1±1.4	29.4±3.6
5	54.8±1.9	15.6±1.5	70.4±2.9	21.8±1.6	29.6±2.9
6	46.8±1.2	10.5±1.3	57.3±2.1	17.9±1.8	42.7±2.1
Dark					
1	15.7±2.5	6.3±1.6	22.1±3.9	25.7±4.2	77.9±3.9
2	16.5±2.0	3.0±0.9	19.5±2.5	13.6±3.8	80.5±2.5
3	32.5±5.1	8.4±2.0	40.8±6.9	19.3±2.4	59.2±6.9
4	25.7±2.4	6.2±1.2	31.9±3.3	18.8±2.3	68.1±3.3
5	25.8±4.8	4.4±1.4	30.2±6.0	11.9±2.8	69.8±6.0
6	14.2±2.6	0.9±0.5	15.2±3.1	4.1±1.6	84.8±3.1
			12-h Means		
Baseline					
Light	56.5±1.1	12.4±0.8	68.9±1.5	17.9±1.0	31.1±1.5
Dark	21.9±1.6	5.0±0.5	26.9±1.9	18.5±1.2	73.1±1.9
Deprivation					
Light	1.4±0.3	0.0±0.0	1.4±0.3	0.0±0.0	98.6±0.3
Dark	5.1±0.4	0.0±0.0	5.1±0.4	0.0±0.0	94.9±0.4
Recovery 1					
Light	61.0±0.5*	23.9±0.8*	84.9±0.7*	28.1±0.7*	15.1±0.7*
Dark	33.7±1.1*	8.1±0.5*	41.9±1.3*	19.4±0.9	58.1±1.3*
Recovery 2					
Light	60.1±1.2*	10.9±0.6†	71.0±1.6†	15.3±0.6*	29.0±1.6†
Dark	28.3±1.4*	6.1±0.4†	34.4±1.6*	17.7±1.1	65.6±1.6*
			24-h Means		
Baseline	39.2±4.1	8.7±1.0	47.9±5.0	18.2±0.7	52.1±5.0
Deprivation	3.2±0.5	0.0±0.0	3.2±0.5	0.0±0.0	96.8±0.5
Recovery 1	47.4±3.2*	16.0±1.9*	63.4±5.0*	23.8±1.2*	36.6±5.0*
Recovery 2	44.2±3.8*	8.5±0.7	52.7±4.3*	16.5±0.7*	47.3±4.3*

Values are means ± SE of %artifact-free recording time; $n = 10$ rats. NREM, non-rapid eye movement; REM, rapid eye movement; TS, total sleep. Significant differences from corresponding baseline values by 2-tailed paired t test: * $P < 0.01$; † $P < 0.05$.

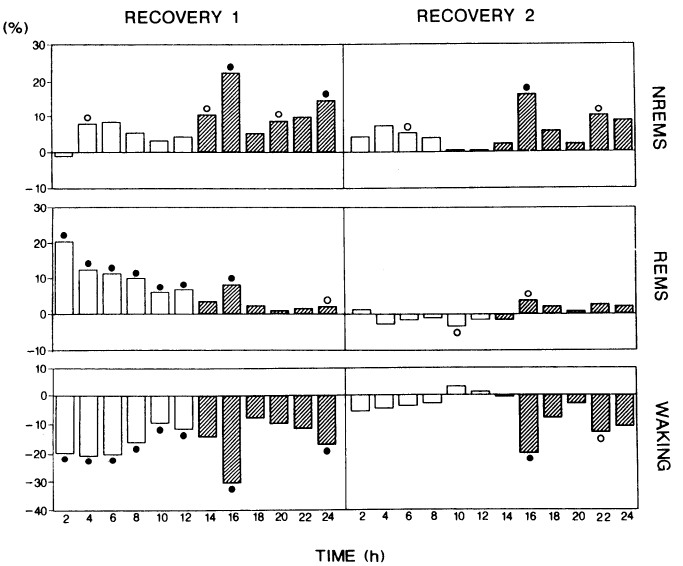

FIG. 2. Vigilance states during 2 days of recovery. Bars represent mean differences from corresponding 2-h baseline values (expressed as percentage of artifact-free recording time, $n = 10$) in light (open bars) and dark periods (crosshatched bars). Significant differences from baseline by 2-tailed paired t test: $^{\circ}P < 0.05$; $^{\bullet}P < 0.01$.

TABLE 2. *Summary of statistical analysis of effect of factors "day" and "2-h interval" on EEG spectrum and their interaction*

	Day	2-h Interval	Interaction
df	2	11	22
NREM Sleep	0.25–25.0	0.25–25.0	0.25–7.0
REM Sleep	3.25–25.0	1.25–9.0	
		10.25–15.0	
		18.25–25.0	
Waking	0.25–25.0	0.75–1.5	
		2.75–7.0	
		9.25–14.0	
		16.25–22.0	

Values are frequency ranges in Hz in which significant effects were present ($P < 0.01$ by 2-way ANOVA); $n = 9$ rats.

period than in the light period. REM sleep values were slightly below baseline in the light period and slightly above baseline in the dark period.

The effect of SD on sleep continuity was analyzed by calculating the *n*BA in TS per 4-h interval. In the baseline light period, the mean *n*BA was 15.3 ± 1.4/TS, and in the dark period it was 16.9 ± 1.0/TS. The values of recovery were expressed as a percentage of corresponding baseline values (see Fig. 8). In the first 4 h of *recovery 1*, *n*BA was significantly reduced. Thereafter *n*BA increased, exceeding baseline in the fifth and some later 4-h intervals, and reverted toward baseline in the dark period of *recovery 2*.

Spectral analysis of the EEG. To examine the effects of experimental day and time of day on the EEG spectrum, the 2-h mean power density of each frequency bin within the individual vigilance states was statistically analyzed by use of a 3 (day: baseline and *recoveries 1* and *2*) by 12 (2-h interval: 1–12) ANOVA (Table 2). The factor "day" was significant for all frequency bins of all three vigilance states except for the lowest bins of REM sleep. The factor "2-h interval" was significant for all frequency bins of NREM sleep and for most bins of REM sleep and waking. The time course in the low NREM sleep frequencies was affected by the experimental condition as indicated by the significant interaction of day and 2-h interval.

In the light period of baseline, power density in NREM sleep showed a decreasing trend in the low frequencies (<7.0 Hz) and an increasing trend in the higher frequencies (>8.25 Hz; Fig. 3, *top left*). In the dark period no trend was present in the low-frequency range, but a decreasing trend prevailed in the high-frequency range (14.25–19.0 Hz; Fig. 3, *top right*). At the light-dark transition the values in all frequencies increased. The time course of SWA is also shown in Fig. 7. Power density in waking and REM sleep showed no significant variation in baseline (data not shown).

During SD, power density in waking increased gradually over the initial 18 h and then remained at an elevated level (Fig. 4, *left*) in most frequency bins. Because of the rare occurrence of NREM sleep in the light period of the

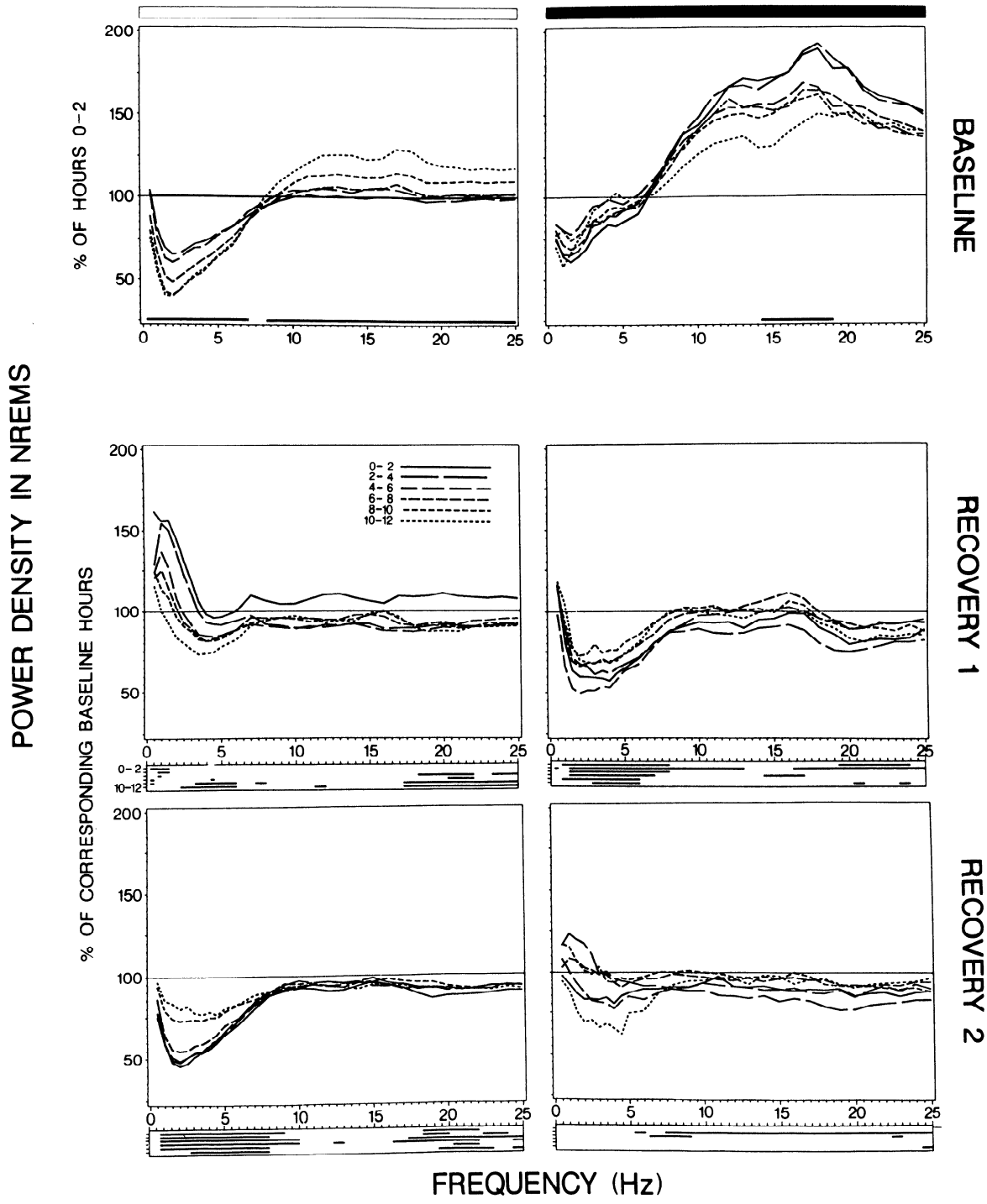

FIG. 3. Time course of EEG power density in NREMS for baseline and recovery. *Left*: light periods. *Right*: dark periods. Curves connect geometric means of relative power density for consecutive 2-h intervals (n = 9). Values are computed for 0.5- or 1.0-Hz bins (see METHODS AND EXPERIMENTAL PROCEDURES) and plotted at upper limit of each bin. *Top*: baseline day. Two-hour mean power density in each bin is expressed relative to its value in first 2 h (= 100%) of light period. Line above abscissa indicates frequency bins in which factor "2-h interval" was significant ($P < 0.01$, one-way ANOVA). *Middle*: 1st day of recovery. *Bottom*: 2nd day of recovery. Data are expressed relative to corresponding 2-h interval (= 100%) of baseline. Lines below abscissas indicate for consecutive 2-h intervals (*top* to *bottom*) those frequency bins in which power density differed significantly from corresponding baseline value ($P <$ 0.01 by 2-tailed paired t test on log-transformed values).

SD day, only few 8-s epochs contributed to the mean power density in NREM sleep (*hours 0–6*: n = 7, mean 1.1 min; *hours 6–12*: n = 9, mean 7.6 min). NREM sleep became more frequent in the dark period (*hours 12–18*: n = 9, mean 17.3 min; *hours 18–24*: n = 9, mean 18.1 min). The spectra showed in almost all frequencies an increase over the first two 6-h intervals (Fig. 4, *right*; see Fig. 7 for SWA). However, the values in the lowest frequencies remained below the reference level.

In *recovery 1* a significant increase in NREM sleep

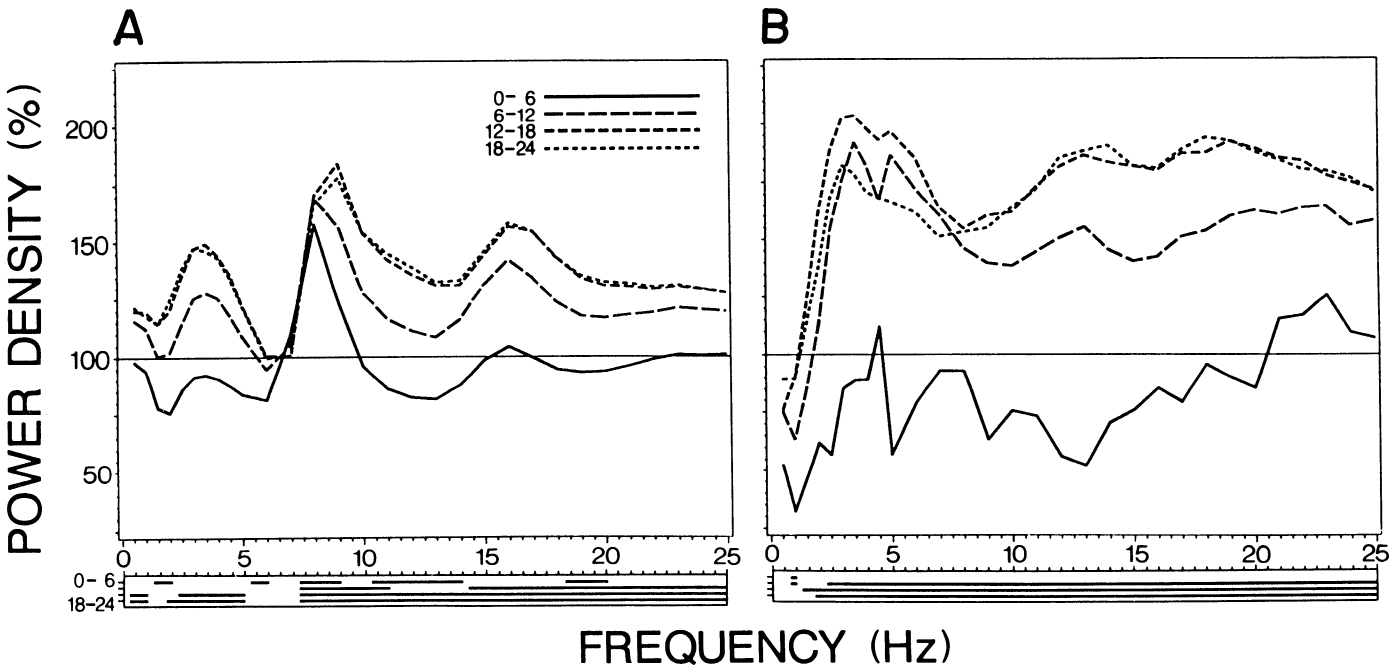

FIG. 4. Time course of EEG power density in waking (*A*) and NREMS (*B*) during sleep deprivation day. Curves connect geometric means of relative power density for 6-h intervals ($n = 9$). Data are expressed relative to mean power density of baseline day (= 100%) in each vigilance state. Lines below abscissas indicate for consecutive 6-h intervals (*top* to *bottom*) those frequency bins in which power density differed significantly from reference value ($P < 0.01$ by 2-tailed paired t test). See legend of Fig. 3 for details.

power density was present in the 0.25- to 1.5-Hz band in the first 2-h interval (Fig. 3, *middle left*). This effect gradually subsided, and the changes were progressively restricted to the lowest frequencies. SWA was increased in the first 4 h of *recovery 1* (see Fig. 7). A reduction below baseline in the 3.25- to 6.0-Hz band was present in the second half of the light period. SWA was significantly below baseline in the last 2 h of the light period. It is evident from Fig. 3 that the negative deviation from baseline became gradually larger and encompassed an increasing frequency range. This negative deviation remained prominent in the dark period (Fig. 3, *middle right*; see Fig. 7). A reduction of power density in the frequencies >18.25 Hz was present in the last 8 h of the light period and in the first 4 h of the dark period. In the dark period there was no consistent trend over successive intervals, although in the 0.75- to 6.0-Hz band the differences from baseline decreased.

Power density in waking was significantly elevated during *recovery 1* in most frequency bins (Fig. 5, *top left*). Power density in REM sleep was elevated in the light period in the 6.25- to 25.0-Hz range and in the first 6-h interval also in some lower frequencies (Fig. 5, *top right*). In the dark period the values reverted toward baseline except for the 7.25- to 9.0-Hz range.

In *recovery 2* power density in NREM sleep remained below baseline during the entire light period over a large range of low (0.75–9.0 Hz) and high (18.25–25.0 Hz) frequencies (Fig. 3, *bottom left*; see Fig. 7). In the low-frequency range the difference with baseline became smaller in the last 4 h. In the dark period the values differed little from baseline with the exception of a decrease in the high frequencies in the second 2 h (Fig. 3, *bottom right*). Power density in waking and REM sleep reverted to baseline in *recovery 2* with the exception of the REM sleep values between 7.25 and 8.0 Hz, which remained elevated (Fig. 5, *bottom*).

T_{CRT}. To examine the effect of experimental day and time of day on the mean T_{CRT} as well as on the T_{CRT} within the individual vigilance states, the 2-h T_{CRT} values were statistically analyzed by use of a 3 (day: baseline and *recoveries 1* and *2*) by 12 (2-h interval: 1–12) ANOVA with repeated measures on both factors. The effect of "day" and "2-h interval" was significant for the mean T_{CRT} as well as for the T_{CRT} in the individual vigilance states ($P < 0.01$). Furthermore the interaction between day and 2-h interval was significant.

In baseline a prominent 24-h modulation of T_{CRT} was present with a sharp decrease at lights on and increase at lights off (Fig. 6, *top*). The 12-h mean values in the light period were significantly lower than in the 12-h dark period (Table 3). T_{CRT} within the vigilance states differed significantly. In the light period, T_{CRT} was lowest in NREM sleep, intermediate in REM sleep, and highest in waking. In the dark period, the difference between T_{CRT} in waking and NREM sleep was larger than in the light period, whereas the NREM sleep and REM sleep values were no longer different.

T_{CRT} was elevated during the entire SD (Fig. 6, *bottom*). Mean T_{CRT} increased in the course of the light period, remained high for the first 6 h of the dark period, and then decreased progressively. During the occasional NREM sleep episodes, T_{CRT} decreased sharply (Fig. 1, Table 3); in the dark period mean T_{CRT} was significantly lower in NREM sleep than in waking ($P < 0.01$ by 2-tailed paired t test).

In *recovery 1* the 24-h mean and the two 12-h means of T_{CRT} were below baseline for all three vigilance states

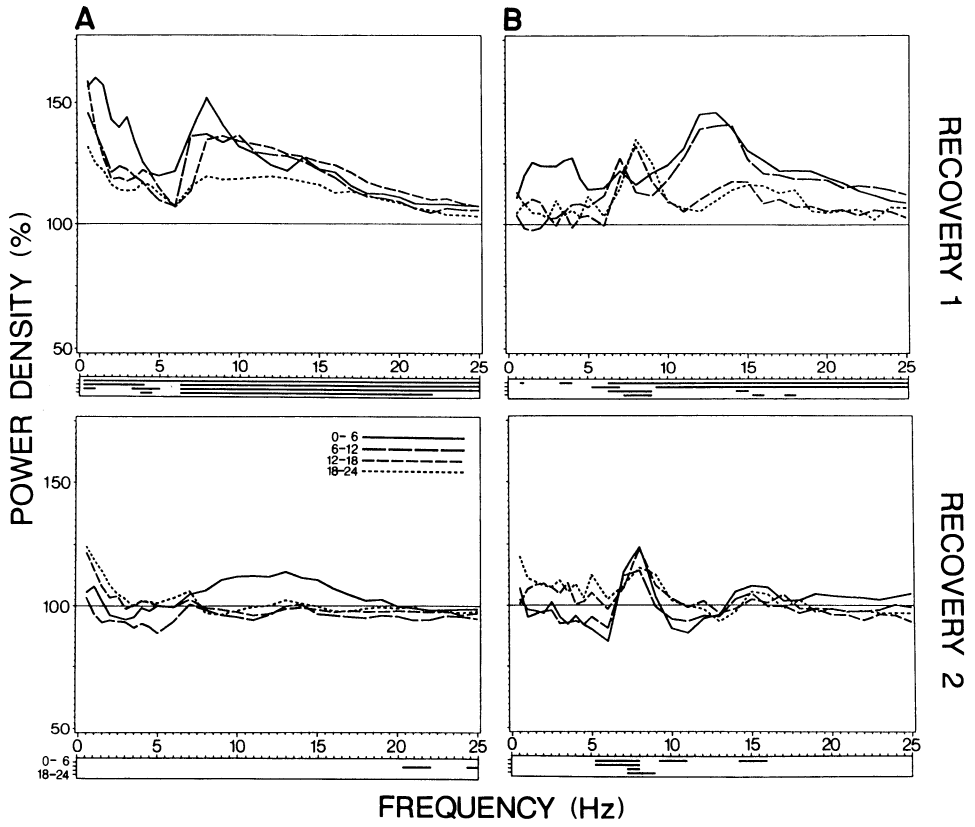

FIG. 5. Time course of EEG power density in waking (*A*) and REMS (*B*) in 1st (*top*) and 2nd day of recovery (*bottom*). Curves connect geometric means of relative power density for 6-h intervals ($n = 9$). Data are expressed relative to corresponding 6-h interval (= 100%) of baseline. Lines below abscissas indicate for consecutive 6-h intervals (*top* to *bottom*) those frequency bins in which power density differed significantly from reference value ($P < 0.01$ by 2-tailed paired t test). See legend of Fig. 3 for details.

(Table 3). After the end of SD, T_{CRT} decreased rapidly and was below baseline in the second 2-h interval (Fig. 6, *bottom*). This decrease was present up to the second 2-h interval of the dark period. The differences of T_{CRT} between the three vigilance states were similar to those of baseline. In *recovery 2*, T_{CRT} had reverted to baseline.

The time courses of SWA in NREM sleep, T_{CRT}, and NREM sleep are summarized in Fig. 7. The curves for NREM sleep and T_{CRT} are almost mirror images over the entire experiment. Moreover, the intervals with a significant difference from baseline (Fig. 7, *top*, horizontal lines) coincide largely for the two variables. In contrast, the time courses of T_{CRT} and SWA in NREM sleep were clearly different. In baseline, SWA gradually decreased in the course of the light period, whereas T_{CRT} declined within the first 2 h and remained at a constant level thereafter. In *recovery 2*, SWA was still significantly below baseline at a time when T_{CRT} had reverted to baseline.

Statistical evaluation of the relationship of the individual 2-h values of NREM sleep and T_{CRT} in baseline and recovery revealed a high negative correlation (product-moment correlation; baseline: $r = -0.82$, $P < 0.001$, $n = 108$; recovery: $r = -0.79$, $P < 0.001$, $n = 214$; baseline + recovery: $r = -0.80$, $P < 0.001$, $n = 322$; Fig. 7, *bottom left*). The absolute SWA in NREM sleep was not significantly correlated with T_{CRT} ($r = 0.02$, $P = 0.79$, $n = 322$). Also, when the correlations were performed with SWA expressed relative to the first 2 h of baseline to minimize the interindividual variation, only a small percentage of the variation in SWA could be accounted for by the variation in T_{CRT} (baseline: $r = 0.24$, $P < 0.05$, $n = 108$; recovery: $r = 0.18$, $P < 0.01$, $n = 214$; baseline + recovery: $r = 0.18$, $P < 0.01$, $n = 322$; Fig. 7, *bottom right*). Furthermore we examined the relationship between T_{CRT} during SD and SWA in NREM sleep shortly after SD. For this we correlated the mean T_{CRT} in the last 2 and 4 h of SD with the mean SWA in NREM sleep in the first 2 h of *recovery 1*. No significant correlation was found (last 2 h T_{CRT} vs. first 2 h SWA: $r = 0.38$, $P = 0.31$, $n = 9$; last 4 h T_{CRT} vs. first 2 h SWA: $r = 0.52$, $P = 0.15$, $n = 9$).

The time courses of SWA in NREM sleep and *n*BA in TS in recovery are illustrated in Fig. 8 (both variables were expressed as a percentage of the corresponding 4-h value in baseline). Both curves presented a similar course. This association was supported by a negative correlation between SWA and *n*BA (product-moment correlation: $r = -0.52$, $P < 0.01$, $n = 108$) and by the overlap of the intervals with significant differences (Fig. 8, horizontal lines).

DISCUSSION

The amount and distribution of the vigilance states (3, 4, 6, 10, 11, 13, 16–18, 22, 23), the time course of the EEG power density (4, 18, 23), and the T_{CRT} values in NREM sleep, REM sleep, and waking (13) are in accordance with previous findings.

Sleep deprivation by handling was successful; NREM sleep was reduced by 92% and REM sleep by 100%. The rising sleep pressure was evident from the larger number of "sleep attempts" in the second 12-h period compared with the first 12 h. Spectral analysis of the waking EEG also revealed a gradual increase in power density in the first three 6-h intervals. A similar trend was seen in

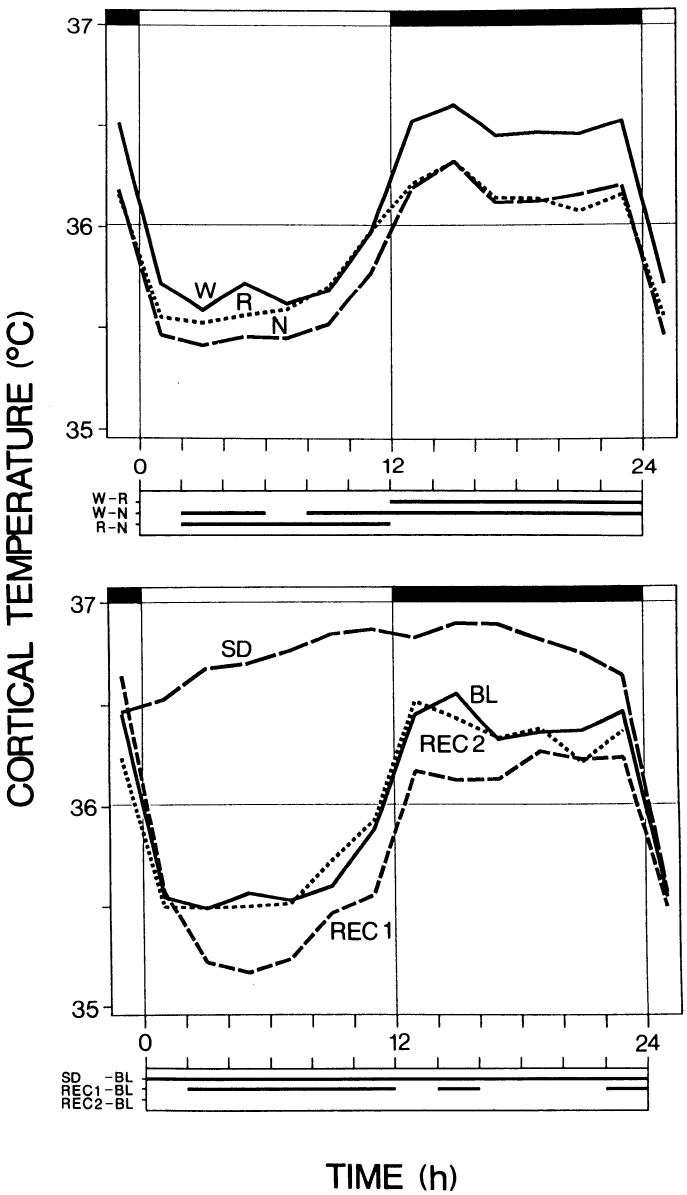

FIG. 6. T_{CRT} during baseline day (*top*) and 4 experimental days (*bottom*). Lines connect mean values for 2-h intervals ($n = 10$) plotted in middle of each 2-h interval. Horizontal line, baseline mean T_{CRT} (36.01°C). *Top*: baseline day plotted for 3 vigilance states separately. Values of first and last 2-h intervals are plotted twice to visualize dark-to-light transition. Lines below abscissa indicate significant differences between vigilance states ($P < 0.05$ by 2-tailed paired t test; SE varied between 0.07 and 0.13°C). *Bottom*: BL, baseline; SD, sleep deprivation; REC1, 1st day of recovery; REC2, 2nd day of recovery. Last 2-h values of each day are plotted twice to visualize dark-to-light transitions. Lines below abscissa indicate significant differences from corresponding 2-h intervals of baseline day ($P < 0.01$ by two-tailed paired t test; SE varied between 0.07 and 0.12°C).

power density of NREM sleep, which reached a plateau already in the second half of the light period. The early plateau may be because the values are derived from very short NREM sleep episodes in which power density could not fully develop (23).

In a study in which the EEG during SD was quantified by period amplitude analysis, the EEG amplitude in waking increased during SD (6). In humans prolonged waking enhanced the EEG power density during waking in delta, theta, and alpha frequencies (20). Thus, in both

TABLE 3. *Mean cortical temperature over 12- and 24-h intervals for individual vigilance states and total recording interval*

	NREM Sleep	REM Sleep	Waking	Total
		12-h Means		
Baseline				
Light	35.5±0.1	35.7±0.1	35.7±0.1	35.6±0.1
Dark	36.2±0.1*	36.2±0.1*	36.5±0.1*	36.4±0.1*
Deprivation				
Light	36.6±0.1†		36.7±0.1†	36.7±0.1†
Dark	36.7±0.1*†		36.8±0.1†	36.8±0.1†
Recovery 1				
Light	35.3±0.1†	35.4±0.1†	35.5±0.1†	35.4±0.1†
Dark	36.0±0.1*†	36.0±0.1*†	36.3±0.1*†	36.2±0.1*†
Recovery 2				
Light	35.5±0.1	35.7±0.1	35.7±0.1	35.6±0.1
Dark	36.2±0.1*	36.2±0.1*	36.5±0.1*	36.4±0.1*
		24-h Means		
Baseline	35.9±0.1	35.9±0.1	36.1±0.1	36.0±0.1
Deprivation	36.6±0.1†		36.8±0.1†	36.8±0.1†
Recovery 1	35.7±0.1†	35.7±0.1†	35.9±0.1†	35.8±0.1†
Recovery 2	35.9±0.1	35.9±0.1	36.1±0.1	36.0±0.1

Values are means ± SE in °C; $n = 10$ rats. $P < 0.01$ by 2-tailed paired t test: * light vs. dark value of each day; † deprivation and recovery values vs. corresponding baseline value.

rat and human, this increase in EEG power density parallels the rising sleep pressure during prolonged waking.

Although the effects of 24 h of SD on the amount and distribution of the vigilance states in the rat have been described in several studies (3, 4, 6, 11, 16, 17, 22), differences in the protocols render a strict comparison of the results difficult. In the only other study in which sleep was prevented by gentle handling for 24 h, the increases in REM sleep and NREM sleep on the 1st day of recovery were comparable (16). Unfortunately a more detailed comparison with the present results is not possible because only 24-h mean values of the 2 days of recovery were presented. Nevertheless, in all other studies an increase in REM sleep was reported. Compared with the studies in which recovery started at the beginning of the 12-h rest period (3, 4, 6, 17), the increase in REM sleep was larger (184% in *recovery 1*); thus the amount of REM sleep lost during SD was almost fully recovered within 1 day. It has been proposed that active waking can substitute for REM sleep (4); thus after SD by forced locomotion the actual REM sleep deficit might be smaller than after SD by gentle handling.

In all previous studies, irrespective of the circadian phase at which recovery began, NREM sleep was decreased in the 12-h rest period of the 1st day of recovery and increased in the 12-h activity period. In contrast, in the present study a significant increase also occurred in the 12-h rest period. However, the relative increase of NREM sleep was more prominent in the 12-h activity period. The rebound of NREM sleep persisted on *recovery 2*, with the largest increase again present in the dark period. In previous studies in which a 2nd day of recovery was recorded, no consistent changes in NREM sleep were reported for that day (3, 6, 11, 16, 22).

A rebound in NREM sleep seems to occur preferably in the 12-h dark or activity period, in which under

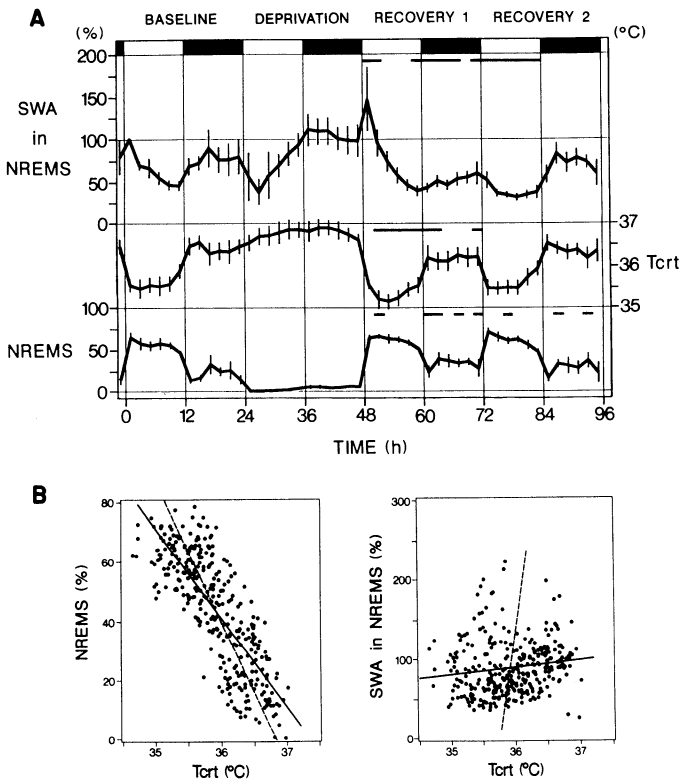

FIG. 7. *A*: time course of SWA (mean power density 0.75–4.0 Hz) in NREMS, T_{CRT}, and NREMS. Experimental condition and dark periods (black bars) are indicated at *top*. Curves connect mean values for 2-h intervals ± 2 SEs (SWA in NREMS, $n = 9$; T_{CRT} and NREMS, $n = 10$) plotted in middle of each 2-h interval. SWA is expressed relative to value in first 2 h of baseline (= 100%). Values of last 2 h of baseline are plotted twice. Significant differences from corresponding baseline values during recovery are indicated by a horizontal line above each curve ($P < 0.05$ by 2-tailed paired t test). *B*: correlation between T_{CRT} and amount of NREMS (*left*) and between T_{CRT} and SWA in NREMS (*right*). Dots represent individual 2-h values from 9 individual rats in baseline and *recoveries 1* and *2*. Lines were fitted to data points with linear regression [T_{CRT} as dependent (dashed lines) or independent variable (solid lines)].

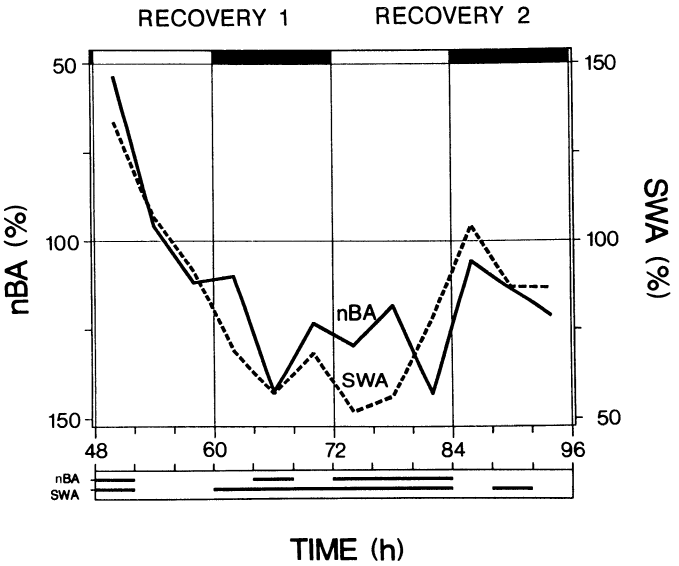

FIG. 8. Time course of SWA in NREMS and of number of brief awakenings (*n*BA; expressed relative to total sleep) in 2 days of recovery. Curves connect geometric mean values over 4-h intervals ($n = 9$) plotted in middle of each 4-h interval. Values are expressed relative to corresponding 4 h (= 100%) of baseline (note reversed scaling of *n*BA). Experimental condition and dark periods (black bars) are indicated at *top*. Significant differences from corresponding baseline values are indicated by horizontal lines below abscissa ($P < 0.05$ by 2-tailed paired t test). SE ranges: SWA 3.1 (*interval 7*) −14.9% (*interval 1*); *n*BA 4.4 (*interval 1*) −22.6% (*interval 6*).

baseline conditions the amount of NREM sleep is lower than in the light period. To account for the absence of a rebound in the light period, Mistlberger et al. (11) postulated an upper limit of TS ("ceiling" effect) of ~80%/6-h interval. However, our results show that TS can reach higher values (in the first 6 h of recovery 91.3% ± 0.9) and that both NREM and REM sleep can increase above baseline level.

In conclusion, the basic features of the recovery pattern of the vigilance states are consistent with earlier findings. The major differences are the larger REM sleep rebound and the earlier NREM sleep rebound, resulting in a marked reduction in waking. These differences may be attributed to the deprivation method, because in most other studies forced locomotion was used.

The loss of NREM sleep was only partly compensated during the 2 days of recovery. However, a NREM sleep deficit may be recovered not only in terms of time spent in this stage but also by an increase in NREM sleep intensity. There is evidence that the power density in the delta frequencies (0.75–4.0 Hz, SWA) is a measure of NREM sleep intensity. In previous studies, a 24-h SD induced an increase in the amplitude or power density in the low-frequency fraction of the NREM sleep EEG during recovery (3, 4, 6, 17, 22). Also, when the SD period was shorter than 24 h (10, 17, 18), a prominent SWA rebound was observed. The present study confirmed the SWA-enhancing effect of SD. However, in contrast to previous studies (4, 18), only the power density in the low delta-frequency range (0.25–1.5 Hz) contributed to this increase. Similar results from a previous study (4) indicate that the SD method may be a crucial variable. In that study sleep was deprived for 24 h by forced locomotion, and subsequently SWS was selectively prevented by handling for an additional 2 h. In the subsequent 6-h recovery period the SWA rebound was lower than after 24 h of SD alone, and the increase in power density was restricted to the lowest-frequency band (0.25–1.5 Hz).

In the present study, the SWA rebound dissipated rapidly; the values decreased significantly below baseline in the last 2 h of the light period of *recovery 1*. This undershoot or negative rebound developed further in the dark period and persisted until the dark period of *recovery 2*. Other authors have reported a negative rebound of EEG amplitude after 24 h of SD (3, 6, 22) and of EEG power density after 12 h of SD (10). This effect was present already within the first 12 h of recovery (10, 22), as in the present study, or only on the 2nd day of recovery (3, 6).

The present analysis revealed that the negative rebound is not a general EEG phenomenon; it is state (power density in NREM sleep was reduced, whereas values in REM sleep and waking were increased on the 1st day) and frequency specific (the main effects were restricted to the frequencies 0.25–9.0 Hz). Furthermore the dynamics of the NREM sleep power spectra within

recovery 1 resembled those under baseline conditions. Previously it has been shown that depriving rats of sleep during a negative rebound still results in an enhancement of SWA (10). Taken together this implies that the homeostatic process, reflected by SWA, is still operative during this period.

The low SWA values in the light period of *recovery 2* were accompanied by a significant enhancement in the amount of NREM sleep. Thus the time course of sleep duration and sleep intensity differed. Sleep discontinuity, represented by the number of brief awakenings, was negatively correlated with SWA. Values were below baseline in the first 4 h of recovery, indicating an increase in sleep continuity, and above baseline afterward, indicating a decrease in sleep continuity. Thus during the negative rebound of SWA sleep was more shallow. This demonstrates that during the entire recovery period SWA is a reliable indicator of sleep intensity.

The question arises whether the negative rebound in SWA is consistent with the homeostatic regulation of SWA, which for humans has been formalized in the two-process model of sleep regulation (2). In this model it is assumed that SWA is regulated by a hypothetical *process S* that depends on the prior history of sleep and waking. During waking *S* increases, and during sleep it decreases. Qualitatively, the time course of SWA in the present experiment may be accounted for as follows: at the beginning of recovery, *S* and thus SWA were at a high level because of SD. Because sleep duration was consistently enhanced throughout the recovery period, *S* declined rapidly and remained for >24 h below the baseline level. It is obvious that a quantitative simulation of the exact time course of SWA would require a definition of the parameters that define the rate of increase of *S* during waking and its decrease during sleep. After the preliminary simulations of Trachsel (21), who assumed that *S* does not change during REM sleep, we were able to simulate the time course of SWA (unpublished results). The results are consistent with the assumption that the negative rebound may indeed be a consequence of the increase in sleep duration after SD. The timing of the negative rebound in previous studies is in accordance with this interpretation. It was postponed to the 2nd day of recovery in those studies in which the amount of NREM sleep was initially not increased (3, 6). Furthermore, an earlier occurrence of the negative rebound was present in studies with 12 (10) or 24 h of SD (22), in which recovery sleep started in the 12-h activity or dark period, and where consequently a relatively large increase in NREM sleep was already present early in the recovery period. Finally, in a study in which waking was increased and NREM sleep decreased after 24 h of SD (4), SWA remained elevated throughout the first 8 h of recovery. Previously the negative rebound has been discussed in terms of "sleep inhibition" and of sleep parameters being controlled by "underdamped servomechanism" (6). In our interpretation, the negative rebound in SWA is a direct consequence of the change in sleep duration.

Sleep deprivation did not only affect the power spectrum in NREM sleep but also enhanced power density in REM sleep in the first 6 h of recovery. High SWA pressure was reflected by the intrusion of slow waves into REM sleep. In the course of *recovery 1* all values reverted to baseline except for theta activity (power density 6.25–9.0 Hz). Theta activity in REM sleep has been proposed as a measure of REM sleep intensity (4). However, in the present study and in two earlier reports (4, 18) theta power did not revert to baseline although the amount of REM sleep did. This shows that the duration and the spectral parameters exhibit a different time course also for REM sleep.

The present data underscore the strong interrelation between sleep duration and T_{CRT}: the amount of NREM sleep was negatively correlated with T_{CRT} throughout the experiment. During SD, the increase of T_{CRT} indicates that the enforced waking induced a thermal load. This thermal load was followed by a decline of T_{CRT} below baseline, an increase in sleep duration, and an intensification of NREM sleep during recovery. However, variations in T_{CRT} during SD did not correlate with the increase in SWA during recovery sleep. In human studies a relationship between temperature and sleep has been found. Horne and Staff (8) reported an increase in SWS (stages 3 and 4; NREM sleep with high SWA) in humans after actively or passively elevating the body temperature and attributed this effect to a thermal load. Berger and associates (1) observed that in humans the duration of SWS correlated with tympanic and rectal temperature at SWS onset, suggesting a direct link between sleep intensity and body temperature. Although our findings are in agreement with the now widely held belief that NREM sleep serves a thermoregulatory function (12, 14, 25) and with the notion that a thermal load induces an intensification of NREM sleep, they failed to confirm a close link between NREM sleep intensity and temperature; the time courses of SWA and T_{CRT} were clearly different, and the thermal load incurred by the SD could not predict the extent of the increase in SWA during initial recovery sleep.

Taken together the data show that SWA covaries with sleep continuity. The present data do not suggest, however, that variations in the intensity of NREM sleep are related to thermoregulatory processes.

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