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Sleep-Deprivation: Effects on Sleep and EEG in the Rat

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Summary. 1. The vigilance states (waking, rapid eye movement (REM) sleep, and non-REM (NREM) sleep), motor activity, food intake and water intake were continuously recorded by telemetry in unrestrained rats. In addition, an amplitude measure and a frequency measure (number of zero-crossings (ZCR) per 10 s) of the telemetered EEG-signal was obtained. The animals were recorded during a control day, then subjected to 12-h or 24-h sleep-deprivation (SD) by means of a slowly rotating cylinder, and subsequently recorded for further 1–2 days. The EEG-parameters were recorded also during SD.

2. On the control day, the EEG-amplitude of NREM-sleep exhibited a decreasing trend in the 12-h light-phase (Figs. 3, 4). The occurence of slow wave sleep (SWS; defined as the NREM-sleep fraction with less than 40 ZCR/10 s) was practically limited to the first part of the light-phase (Figs. 2, 4). Cumulative plots of the zero-crossing bands (Fig. 2) revealed a prominent daily rhythm in the EEG-frequency distribution *within* NREM-sleep.

3. The percentage of NREM-sleep and REM-sleep was little affected by the 12-h SD, but the amount of SWS and the EEG-amplitude of NREM-sleep were increased (Figs. 4, 6). After a 24-h SD period terminating before light-onset, NREM-sleep was reduced and REM-sleep was markedly enhanced (Figs. 4, 6; Table 1). Both the duration and frequency of REM-sleep episodes were increased, and episodes of total sleep prolonged (Table 2). The amount of SWS was significantly more increased after 24-h SD than after 12-h SD, whereas the EEG-amplitude of NREM-sleep was enhanced to a similar extent after both SD-schedules (Tables 1, 3: Fig. 6).

4. After a 24-h SD period terminating before dark-onset, sleep (particularly REM-sleep) was enhanced in the first hours of the dark-phase, yet the usual high activity bouts prevailed in the later part of the dark-phase (Figs. 7, 8; Table 1). The extent and time-course of REM-sleep rebound was similar after the two 24-SD schedules, whereas SWS-rebound was different: SWS exhibited a one-stage rebound when recovery started in the light-phase, and a two-stage rebound when recovery started in the dark-phase (Fig. 9).

5. A comparison of the effects of 12-h SD performed with the usual and with the double cylinder rotation rate, showed only small differences, indicating that forced locomotion was a minor factor in comparison to sleep-deprivation (Fig. 10; Table 1).

6. The daily pattern of SWS on control days, and the marked increase of SWS after SD correspond to the results from other animal and human studies. It is proposed that due to the existence of an intensity dimension, NREM-sleep is finely regulated around its baseline level, and thus may be readily and accurately adjusted to current 'needs', whereas REM-sleep, lacking an apparent intensity gradient, is regulated around a level which is considerably below baseline. Thus, in contrast to NREM-sleep, REM-sleep compensation can occur only by an increase in the time devoted to this state, thereby curtailing the time available for other activities.

Abbreviations: *EEG*, electroencephalogram; *EMG*, electromyogram; *FD*, food intake; *INT-AKT*, integrated motor activity; *LQ*, liquid intake; *NREM*, non-REM (sleep); *REM*, rapid eye movement (sleep); *SD*, sleep deprivation; *SWS*, slow wave sleep; *TS*, total sleep; *ZCR*, zero-crossing

Introduction

Although many aspects of sleep have been successfully investigated in the recent years, the functional significance of sleep is still obscure. A biological recovery function of sleep has been repeatedly postulated, but the evidence for this assumption remains

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circumstantial (see Adam and Oswald, 1977). One experimental approach to this problem is the study of the effects of sleep-deprivation (SD) as it may be surmised that the processes subserved by sleep are enhanced after SD, and thus may be more easily detectable. While the fascination exerted by rapid eye movement sleep (REM-sleep), a substate of sleep, has given rise to many experiments in which a selective REM-sleep deprivation was attempted (see Vogel, 1975, for a review), animal studies using total SD have been rarely undertaken. It seemed therefore reasonable to investigate the effects of SD in the rat, an animal for which the circadian sleep-waking rhythm and the distribution of the vigilance states have been described in detail (Borbély and Neuhaus, 1978a, b). In the present study, the vigilance states were supplemented by amplitude and frequency measures of the EEG which served as more refined state indicators. The duration of the deprivation period and its phase relative to the light-dark cycle, were varied to evaluate the influence of waking time on sleep and EEG-parameters, and to study interactions with the circadian rhythm.

Methods and Experimental Procedure

Animals

Fifteen adult male albino rats of the Sprague-Dawley-Ivanovas (SIV) 50 strain (range of body weight: 250–320 g) were used as experimental animals. They were kept individually in transparent plexiglas tubes (40 × 20 × 18 cm) with plexiglas grid floors, to which they had been adapted prior to the experiments, and had unlimited access to food (rat chow cubes, NAFAG, Switzerland, no. 890) and water.

Experimental Set-up and Procedure

Before and during the recording period the animals lived in a separate room that provided sound-attenuation from environmental noise. Ambient temperature was maintained at 22 °C and controlled to within ±0.3 °C. The animals were kept under a 12 h light – 12 h dark (LD 12:12) schedule (light 10–22 h in Experiments 1 and 3; 5–17 h in Experiment 2). Light was provided by overhead, day-light type neon light tubes. Light intensity at the cage level varied, depending on the direction, between 15 and 28 μW/cm². Measurements were made with an optometer (U.D.T. Model 40) using a radiometric filter and a footcandle diffuser.

Gold-wire electrodes were used to record the cortical EEG (parietal cortex) and neck muscle EMG, a cerebellar electrode serving as a common reference. Operations were performed under pentobarbital anesthesia, and at least 5 days were usually allowed for recovery before the recordings were started. Sleep-deprivation was performed by maintaining the animal in a cylinder that was slowly rotated by motor-driven pulleys. The cylinder consisted of a PVC base disk and a top ring (diameter 30 cm) which were joined by regularly spaced PVC-rods (length of rods 30 cm; rod diameter 10.4 mm; interval between rods 15.5 mm). The rotation rate was usually one rotation per 45 s, except for Experiment 3 where also the double rotation rate was used. At least 2 days before the recording period, the animals were exposed to the rotation condition for a 3 h habituation session at the beginning of the dark-phase. Unlimited access to food and water was provided also in the cylinder.

The following 3 experimental schedules were used:

Experiment 1 (7 days, 6 rats, 12-h and 24-h SD): After a control day starting with light-onset the animal was transferred into the cylinder during the last 30 min of the dark-phase, and kept there without rotation during the 12-h light-phase of day 2. Rotation was started with dark-onset and lasted close to the end of the dark-phase (12-h SD). Subsequently the animal was transferred back into its usual recording cage, and maintained there for 2 days (days 3 and 4). Then the animal was placed again into the cylinder and rotated for 24 h (24-h SD) starting with light-onset. The final 2 days in the recording cage (days 6 and 7) concluded the experiment.

Experiment 2 (4 days, 5 rats, 24-h SD): In this experiment, recovery from 24-h SD was allowed at the beginning of the dark-phase, the rat's usual daily activity phase. After a control day starting with dark-onset, the animal was transferred into the cylinder at the end of the light-phase. Rotation was started with dark-onset and lasted for close to 24-h (24-h SD). Then the animal was transferred back into the recording cage and recorded there for another 2 days.

Experiment 3 (5 days, 4 rats, twice 12-h SD): After a control day starting with light-onset, the animal was transferred into the cylinder and kept there without rotation during the 12-h light-phase of day 2. Rotation at the usual rate (1 rotation per 45 s) was started with dark-onset and lasted close to the end of the dark-phase (12-h SD). The animal was put back into the recording cage and recorded there for 1 day. Then the animal was placed for a second time into the cylinder and maintained there without rotation during the 12-h light-phase of day 4. Rotation at the double rate (1 rotation per 22.5 s) was started with dark-onset and lasted close to the end of the dark phase (12-h SD). The animal was returned to the recording cage and recorded there during the final day (day 5).

In Experiments 1 and 3 all manipulations of the animal were performed under dim red light within the last 30 min of the dark-phase, and in Experiment 2 within the last 30 min of the light-phase. The experiments were carried out in the following months: Experiment 1: July-October; Experiment 2: October-December; Experiment 3: December-January.

Recording and Data Analysis

The following parameters were recorded; the vigilance states: waking, rapid eye movement sleep (REM-sleep = paradoxical sleep); non-rapid eye movement sleep (NREM-sleep); motor activity; food and water intake; the integrated, rectified EEG-signal (INT-EEG); and the zero-crossing rate of the EEG-signal (ZCR-EEG). With the exception of the latter two parameters, the recording methods have been described previously (Neuhaus and Borbély, 1978; Ruedin et al., 1978), and will be only briefly summarized.

Motor activity was recorded by means of a force recorder under the animal's cage. It consisted of a platform resting on springs and moving-coils serving as mechano-electrical transducers. The vertical component of force changes occurring during motor activity induced proportional changes in voltage which were amplified and full-wave rectified to provide the AKT-signal.

The *EEG* and *EMG* were recorded with a miniature battery-

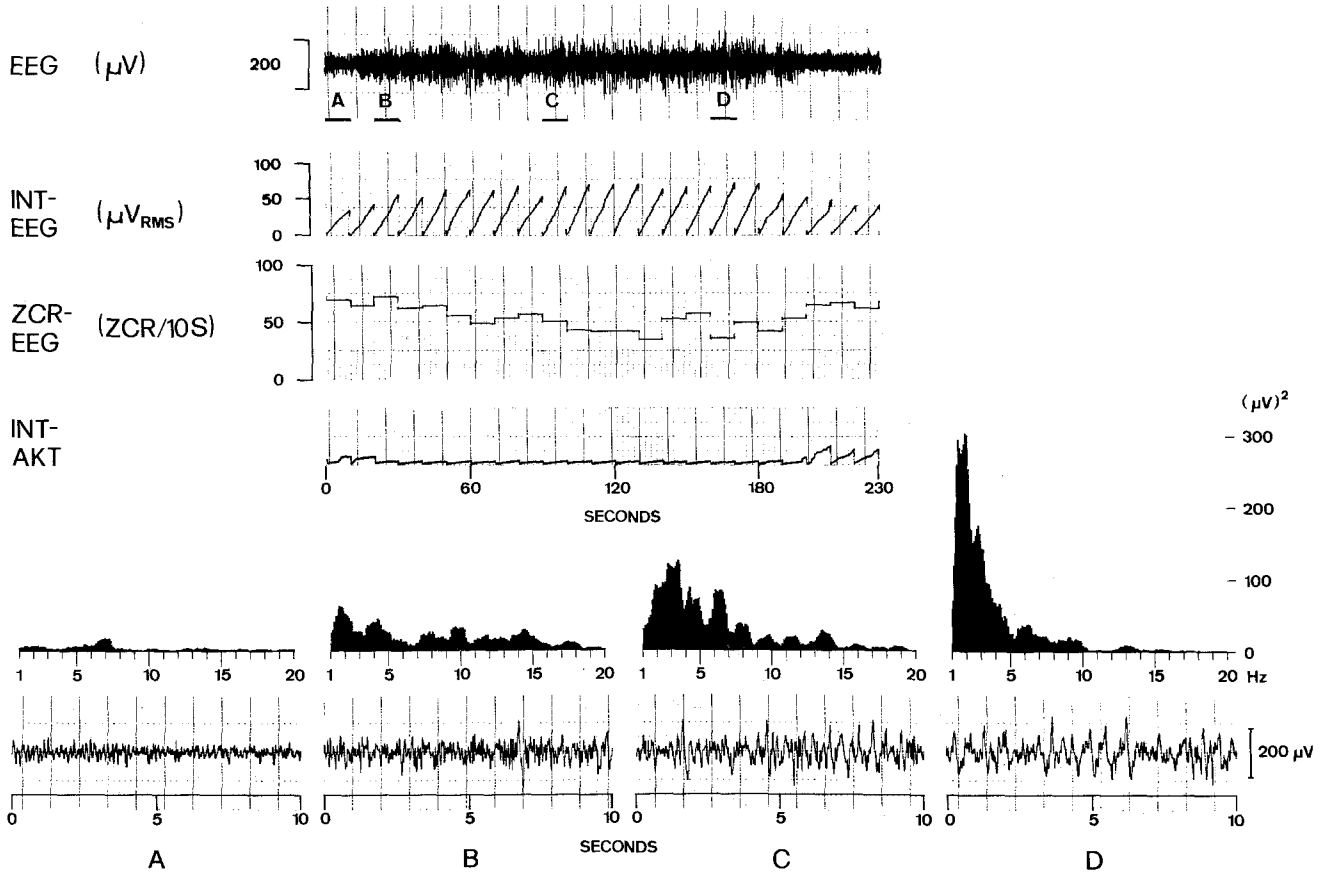

Fig. 1. EEG with power spectra, EEG-parameters and motor activity during a NREM-sleep period recorded 2 h after light-onset. Upper part: EEG recorded at low paper speed; integrated rectified EEG-signal (EEG-amplitude) for successive 10-s periods (INT-EEG); number of zero-crossings per 10-s (ZCR-EEG); and integrated motor activity (INT-AKT). Lower part: EEG of four 10-s periods plotted at high paper speed with corresponding power spectra. Position of the samples is indicated below top EEG-record by letter and bar. A: waking; B: onset of NREM-sleep; C: NREM-sleep with a moderate predominance of low frequencies; D: NREM-sleep with a high predominance of low frequencies (slow wave sleep). Units for upper records are indicated on left side; INT-AKT is plotted in arbitrary units, the minimum level corresponding to zero. The spectra are plotted as moving averages of 10 successive 0.1 Hz values (steps of 0.1 Hz). Thus the value above the 1 Hz mark represents the average of 10 values between 0.5 and 1.4 Hz. The spectra are expressed as $(\mu V_{RMS})^2$ per 0.1 Hz as shown on the right ordinate (animal 31)

powered, 2-channel FM-AM transmitter (weight: 3.0 g) fastened to the animal's head. The signal was received by 3 antennae, demodulated and fed into the state identification circuit.

Food intake (FD) and *Liquid intake* (LQ) was measured by means of mechano-electrical balances which recorded the weight of the food and water containers.

The automatic *state identification system* consisted of an on-line and a off-line stage. The on-line stage determined at 10-s intervals whether the preset threshold values of the following integrated parameters were exceeded or not: AKT; the ratio 'rectified EEG/AKT'; theta activity (6–9 Hz); and the rectified EMG. In addition, the rectified EEG-signal was integrated over a 10-s period to provide the INT-EEG value. The zero-crossing rate per 10-s was obtained by counting the number of times the filtered EEG-signal (low pass, 6 dB at 12 Hz, 12 dB/octave) crossed the zero-volt level in the negative-positive direction. The threshold information for EEG/AKT, theta activity and EMG was stored on paper-tape for successive 10-s epochs together with the values for INT-EEG, ZCR-EEG, FD, LQ and AKT. In the off-line stage, the information stored was read from paper-tape into a PDP-11/20 computer and stored on disk. The threshold values and AKT were used for the identification program of the vigilance states (Neuhaus and Borbély, 1978). The performance of the identification system was checked almost daily by a comparison with visually scored polygraph recordings.

In Experiments 1 and 2, the EEG was recorded by telemetry also during sleep deprivation in the rotating cylinder (Figs. 4, 5). However, the vigilance states were not identified during the SD-periods, because the force-recorder could not be used. Nevertheless, an activity measure was obtained by recording the field changes induced by head-movements, a method yielding signals that are comparable to the force recorder output (Ruedin et al., 1978).

To evaluate the relationship between the ZCR-value and the EEG-power-spectrum, samples of the EEG and AKT-signals of several animals were recorded on analog magnetic tape (Sangamo, Model 3500). Then the EEG-signals were played back from tape, passed through the A/D converter of the PDP-11/20 computer (conversion rate 102.4 Hz) and stored on disk. After weighting the 10-s epochs with a Hanning Window, a discrete Fourier-transformation was used to obtain the power-spectra (Software: Lab Applications-11).

Due to occasional deficiencies in the recording system, data from some animals could be only partially used for the computation of the average values presented in the Results section.

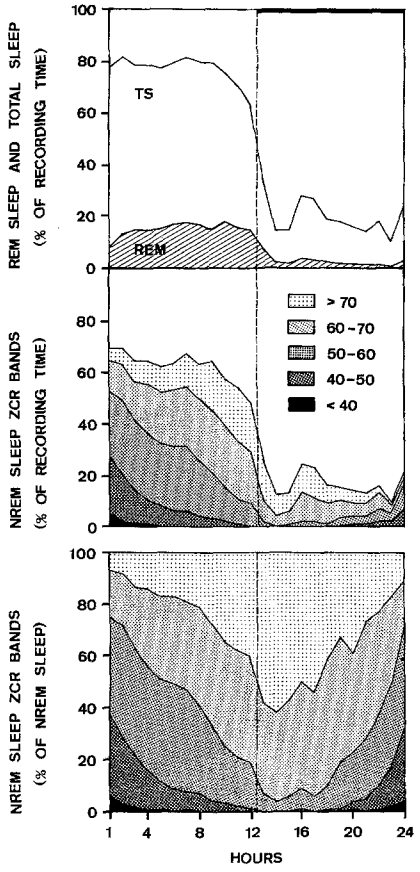

Fig. 2. Daily distribution of sleep states. The curves represent hourly mean values from 15 animals recorded on the control day. Dark bar on top abscissa and interrupted vertical line delimit the dark-phase. *Top:* Total sleep (TS) and REM-sleep (hatched area) expressed as the percentage of recording time. The percentage of waking is represented by the difference between TS and the top abscissa. *Middle:* NREM-sleep (uppermost curve) expressed as the percentage of recording time and subdivided into 5 zero-crossing bands as indicated on the inset (the numbers represent zero-crossing per 10 s). Decreasing values (increasing darkness of area) reflect an increasing predominance of low frequencies in the EEG. *Bottom:* NREM-sleep with zero-crossing bands expressed as the percentage of total NREM-sleep (100%). One-hour periods with less than 5 min NREM-sleep were not used for computing the mean hourly values

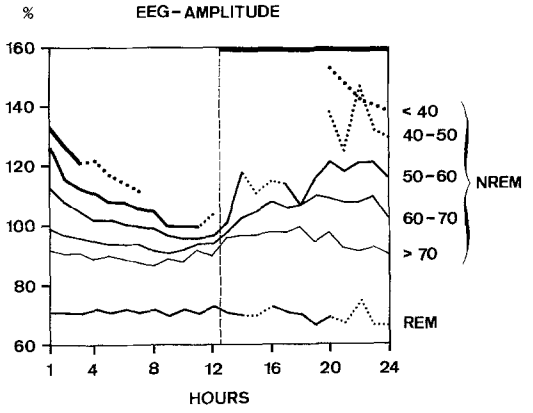

Fig. 3. Daily distribution of EEG-amplitudes of NREM-sleep and REM-sleep. The curves represent hourly mean values from 15 animals recorded on the control day. Dark bar on top abscissa and interrupted vertical line delimit the dark-phase. The amplitude of NREM-sleep was computed for 5 zero-crossing bands as indicated on the right ordinate (the numbers represent zero-crossing per 10 s; curves for increasing values are plotted by lines of decreasing thickness). The lowest curve represents the amplitude of REM-sleep. The dotted lines indicate periods for which less than 4 animals contributed to the mean values. Animals with less than 6 amplitude values per hour in a given ZCR-band were not used for computing the hourly mean value. The amplitudes are expressed as the percentage of the mean daily NREM-sleep amplitude of individual animals

Results

The EEG-Parameters

In addition to recording the vigilance states and motor activity, the following two EEG-parameters were measured for successive 10-s periods: (1) The integrated value of the rectified EEG-signal (INT-EEG; Figs. 1, 4, 7), also denoted as the mean EEG-amplitude (Fig. 3; Table 3); and (2) the number of zero-crossings per 10 s (ZCR-EEG, Figs. 1, 4, 7), a parameter that provides an estimate of the dominant frequency in the EEG-signal. Thus a flat distribution of frequencies in the spectrum yields a high ZCR-value (Fig. 1A,B; Fig. 5: two middle records), whereas the predominance of low frequencies results in a low ZCR-value (Fig. 1C,D; Fig. 5: top and bottom records). The ZCR-values shown above the spectra in Figure 5 illustrate the relationship between the frequency measures obtained by the ZCR-method and by spectral analysis.

The zero-crossing parameter was used to subdivide NREM-sleep into 5 'zero-crossing bands' ranging in steps of 10 units from <40 ZCR/10-s to >70 ZCR/10-s (Fig. 2). The lowest ZCR-band represent the fraction of NREM-sleep with the most marked predominance of slow waves and has been therefore arbitrarily defined in this study as slow wave sleep (SWS). It occurs typically only in the first part of the light-phase. The highest ZCR-band of NREM-sleep (>70 ZCR/10-s) occurs at sleep onset (Fig. 1B), and is typically seen during the frequent alterations of sleep and waking in the last part of the light-phase where NREM-sleep exhibits often a flat frequency distribution in the EEG-spectrum (Fig. 5). Figure 1 illustrates a sequence of NREM-sleep in the morning which is delimited by waking periods. Note that with the third 10-s period (B) movement (AKT) ceases completely and the EEG-amplitude (INT-EEG) in-

creases, while in the spectrum high and low frequencies are present to a similar extent. The consecutive 'deeping' of sleep is reflecting by a further increase in EEG-amplitude and a decrease of the ZCR-values (C). Sample D represents one of two 10-s periods with a maximum predominance of slow waves. The reverse changes are seen before the animal awakens.

Control Day

The top records of Figures 4 and 7 show original computer plots obtained for control days (the cumulative curves of food and fluid intake were usually also plotted, but have been omitted here; see Borbély and Neuhaus, 1978a). During the 12-h light-phase sleep predominates, whereas waking and motor activity prevail during the dark-phase. The two EEG-parameters exhibit clear trends within the light-phase. Thus the EEG-amplitude during NREM-sleep is high after light-onset and then shows a decreasing trend. High amplitude values can be seen also during the sporadic sleep periods in the later part of the dark-phase. The decreasing trend of EEG-amplitudes is evident from the mean values in Table 3 (upper right). In all 15 animals the amplitude of the first 3-h period in the light-phase invariably exceeded the amplitude of the second 3-h period ($P < 0.01$; sign-test).

Figure 3 shows the daily pattern of the EEG-amplitudes for the various ZCR-bands of NREM-sleep and for REM-sleep. The dotted part of the curves represents periods in which less than 4 animals contributed to the hourly mean value, while the gap in two curves (<40 and 40–50 ZCR/10 s) indicates periods for which no values were measured (cf. Fig. 2). The amplitudes in Figure 3 are expressed as the percentage of the mean daily NREM-sleep amplitude of individual animals. Note the inverse relationship between the amplitudes and the ZCR-bands. Yet even the band with the lowest NREM-sleep amplitudes (>70 ZCR/10 s) is still much higher than the amplitude of REM-sleep. The decreasing trend of NREM-sleep amplitude in the early part of the light-phase is most prominent for the low ZCR-bands, and is practically absent in the highest band. This leads to a convergence of the curves in the end of the light-phase. A marked amplitude increase occurs with dark-onset, and a slight increasing trend in the dark-phase may be seen for the 50–70 ZCR-bands. The very high values in the lowest ZCR-bands before light-onset are striking, even though only 1–3 animals contributed to the data points. Considering the NREM-sleep amplitudes irrespective of ZCR-bands, it was seen that for 14 out of 15 animals the values in the dark-phase were higher than in the light-phase ($P < 0.001$; 2-sided paired t-test; see also Table 3). It should be noted, however, that the amplitude changes were not strictly related to the lighting conditions, but represent probably a circadian variation. The EEG-amplitude of REM-sleep exhibited neither a trend during the day (Fig. 3) nor a difference between the lighting phases (Table 3).

The ZCR-values and the EEG-amplitude of NREM-sleep showed inverse trends in the control day. Thus the ZCR-curve was low at the beginning of the light-phase, and then rose gradually (Figs. 4, 7). During the later part of the light-phase, SWS (as defined in the present study) was virtually absent, and a relatively flat frequency distribution was typically seen in the EEG-spectrum (Fig. 5). The average daily distribution of the ZCR-bands of NREM-sleep is illustrated in Figure 2. Note that the total amount of NREM-sleep (middle display, uppermost curve) gives no indication of the frequency shifts that occur within NREM-sleep during a 24-h period. It may be seen from the cumulative ZCR-curves that the lower ZCR-bands exhibit a marked decreasing trend in the light-phase, the lowest band (<40 ZCR/10 s) disappearing early and the adjacent band (40–50 ZCR/10 s) late in the light-phase. Therefore NREM-sleep in the first part of the dark-phase is composed only of the 3 highest ZCR-bands, while in the second part a progressive increase of the 40–60 bands occurs.

In the lowest display of Figure 2 the ZCR-bands are plotted as a percentage of total NREM-sleep to illustrate the frequency shift irrespective of the absolute amounts of NREM-sleep. It is evident that the fraction of NREM-sleep with low ZCR-values decreases to a minimum in the early dark-phase and then shows a progressive, smooth increase. Conversely, the fraction with the highest ZCR-values increases gradually from the early light-phase to the early dark-phase and then decreases again.

Recordings During Sleep-Deprivation

Sleep-deprivation in the rotating cylinder was designed to involve a minimum amount of stress and physical exercise for the animal. Stefurak and coworkers (1977) have described a similar procedure for obtaining SD, but used a higher rotation rate.

In the present study the effectiveness of the SD-procedure could be evaluated from the EEG-amplitude and ZCR-values which were recorded throughout the deprivation period. The lowest display of Figure 4 is an example of a recording obtained during 24-h SD. Note the steady level of the two

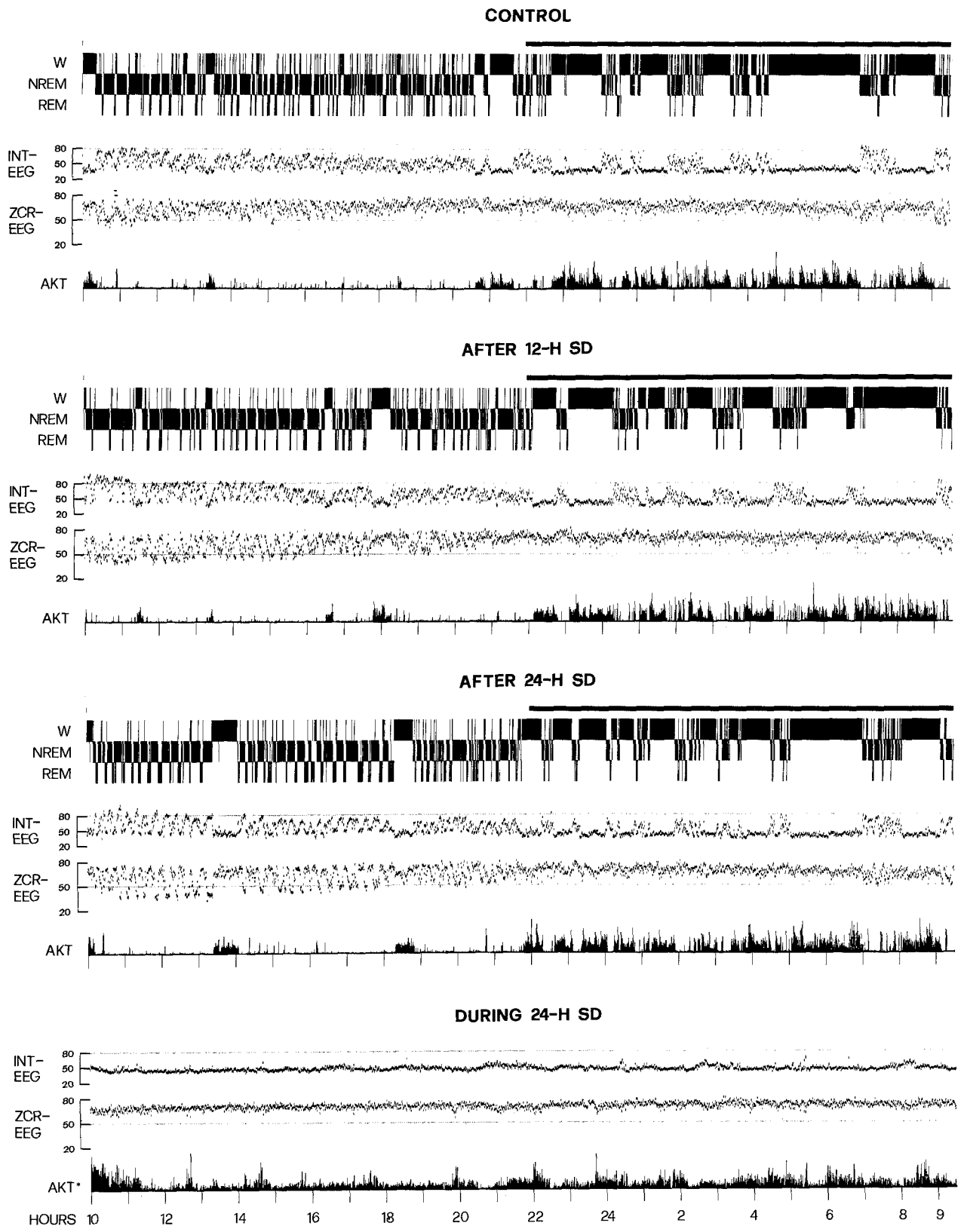

Fig. 4. Vigilance states, EEG-parameters and motor activity of a rat plotted for the control day (top); the day after 12-h sleep deprivation (SD); the day after 24-h SD; and during 24-h SD in the rotating cylinder (bottom). W: waking; NREM: NREM-sleep; REM: REM-sleep. INT-EEG: full-wave rectified EEG-signal expressed as $\mu V_{RMS}/10$ s; ZCR-EEG: number of zero-crossings per 10 s; AKT: motor activity measured with the force recorder; AKT*: motor activity measured by telemetry (see Methods). Motor activity is plotted in arbitrary units, the baseline corresponding to the zero level. All values are plotted for successive 40-s periods. The predominant vigilance state is indicated. Two vigilance states occurring each for 20 s are indicated both for the same time period. EEG-parameters and motor activity represent arithmetic mean values of 4 successive 10 s periods. Dark bars above vigilance states delimit dark-phase. Hours below abscissa denote time of the day (animal 05)

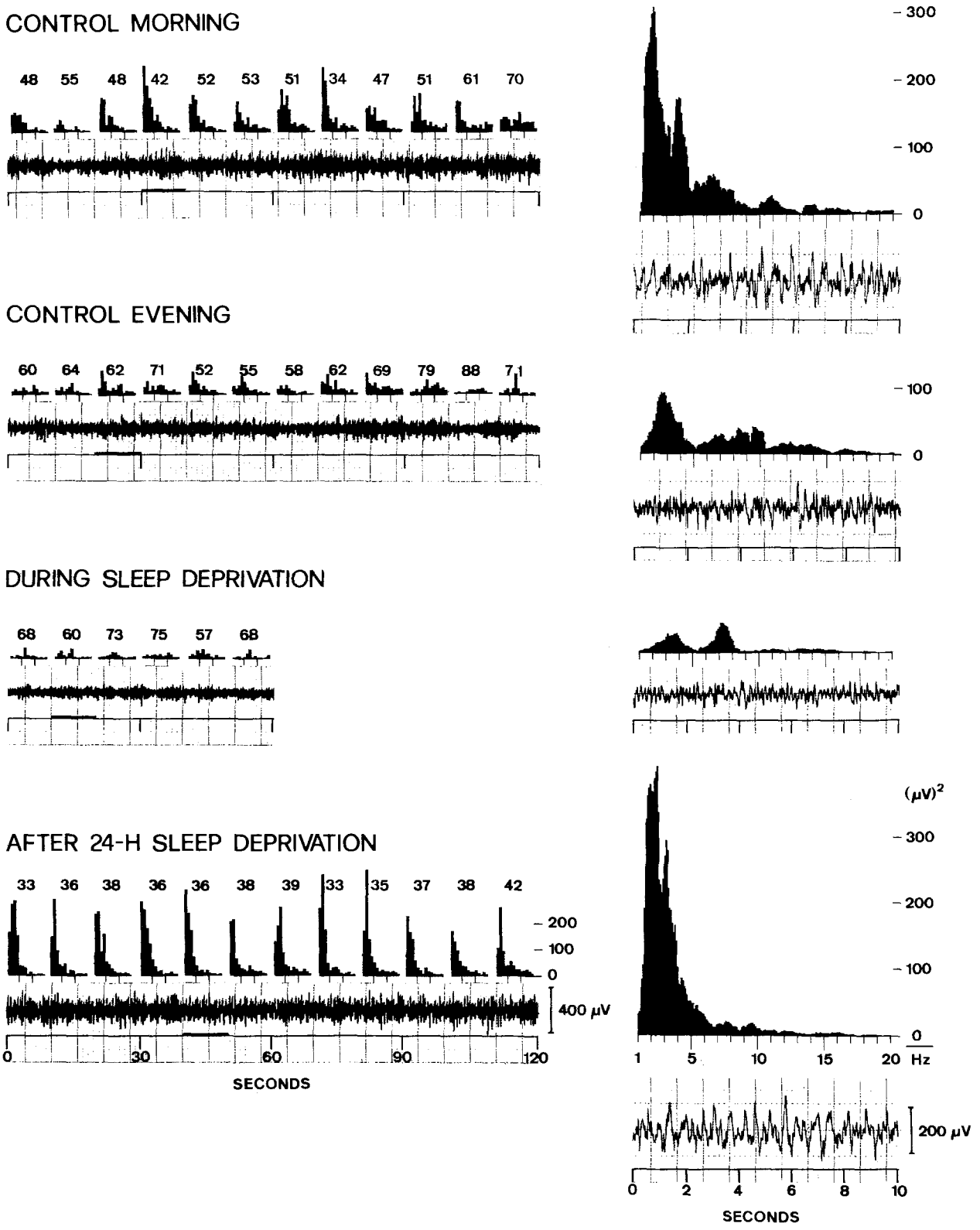

Fig. 5. EEG-records and power spectra obtained from the same animal during sleep in the subjective morning (12:05 h) and subjective evening (20:50 h) of the control day; during sleep-deprivation in the rotating cylinder (14:15 h); and during sleep on the day after 24-h sleep-deprivation (11:50 h). *Left* records: EEG recorded at low paper speed with power spectra (1–15 Hz, plotted in 1 Hz bins) above the corresponding 10-s periods. The number above the spectra represents the number of zero-crossings per 10 s. *Right* records: EEG-samples recorded at high paper speed, selected from the corresponding record on the left side (the selected 10-s period is indicated by bar under EEG). The power spectra corrresponding to the 10-s sample are shown above the EEG-records. They are plotted between 1–20 Hz as moving averages of 10 0.1 Hz values as in Fig. 1. Calibration in $(\mu V)^2$ per 0.1 Hz on the right ordinate. Except for the 1-min sample obtained during sleep-deprivation, motor activity was absent during all recording periods. (Animal 31)

Table 1. Sleep states, motor activity (AKT), food intake (FD) and liquid intake (LQ) for experimental days. Data represent arithmetic mean values (SEM in parenthesis). Sleep states are expressed as percent of recording time, AKT as the percentage of mean motor activity on day 1 for individual animals. L: light-phase; D: dark-phase; TS: total sleep

Experiment	Day	TS		NREM		REM		REM/TS		AKT		FD g/day	LQ g/day
		L	D	L	D	L	D	L	D	L	D		
1	1	75.4 (1.3)	16.3 (3.0)	59.3 (1.6)	14.0 (2.5)	16.1 (0.6)	2.3 (0.6)	21.4 (0.9)	14.2 (2.6)	43.3 (2.7)	159.1 (2.9)	30.6 (1.6)	27.0 (1.7)
	2	12-h sleep-deprivation											
	3	80.3^{b} (0.8)	19.6 (2.7)	62.5 (1.8)	16.9 (2.2)	17.8 (1.1)	2.7 (0.6)	22.2 (1.5)	13.3 (1.5)	38.6 (1.2)	159.6 (3.7)	32.6 (1.6)	27.6 (2.4)
	4	74.8 (1.3)	19.3 (3.4)	58.7 (0.9)	16.1 (2.7)	16.1 (1.4)	3.2 (0.8)	21.4 (1.7)	15.9 (2.8)	47.9 (4.5)	168.5^{b} (3.2)	32.5 (1.8)	27.3 (2.9)
	5	24-h sleep-deprivation											
	6	80.1 (2.3)	27.0 (4.3)	54.7^{b} (1.4)	21.8 (3.4)	25.4^{d} (1.4)	5.2^{a} (1.0)	31.6^{d} (1.1)	18.9^{b} (1.1)	45.0 (5.7)	156.3 (5.9)	35.0 (2.4)	30.0 (3.2)
	7	75.7 (1.0)	20.1 (2.3)	60.3 (1.5)	16.4 (2.1)	15.4 (0.8)	3.6^{c} (0.3)	20.3 (1.2)	18.7 (1.6)	43.8 (3.3)	175.8^{b} (6.0)	28.5 (1.7)	29.0 (3.8)
3	1	76.9 (1.0)	19.6 (1.9)	64.2 (1.2)	17.7 (1.9)	12.7 (0.5)	1.9 (0.7)	16.5 (0.7)	9.8 (3.6)	44.2 (3.5)	158.2 (3.6)	26.6 (1.3)	36.0 (4.2)
	2	12-h sleep-deprivation: usual rotation rate											
	3	82.5^{a} (1.9)	22.1 (2.1)	70.1 (2.6)	19.7 (1.7)	12.4 (2.0)	2.4 (0.7)	15.1 (2.4)	10.4 (2.4)	37.7 (0.9)	170.2^{b} (5.2)	25.5 (1.9)	33.8 (5.7)
	4	12-h sleep-deprivation: double rotation rate											
	5	77.1^{B} (2.0)	25.4^{a,B} (1.5)	64.3 (1.3)	21.9^{B} (1.5)	12.8 (1.5)	3.5^{a} (0.5)	16.5 (1.7)	13.8 (1.6)	46.7^{A} (3.0)	161.1 (9.4)	24.0 (1.2)	33.8 (1.0)
		D	L	D	L	D	L	D	L	D	L		
2	1	22.6 (3.4)	78.5 (1.7)	19.5 (3.3)	64.1 (1.8)	3.0 (0.5)	14.4 (0.8)	14.1 (2.5)	18.4 (1.0)	161.0 (3.4)	36.3 (3.5)	28.5 (3.0)	35.6 (2.2)
	2	24-h sleep deprivation											
	3	36.8^{b} (2.6)	81.2 (2.5)	24.8^{a} (2.6)	65.0 (2.2)	12.0^{d} (0.9)	16.2^{b} (0.8)	33.0^{b} (2.9)	20.0^{b} (0.8)	135.5 (12.1)	31.9 (3.0)	24.9^{b} (3.0)	30.8 (2.2)
	4	20.9^{a} (2.9)	79.0 (0.4)	18.4 (2.7)	63.9 (0.6)	2.5 (0.5)	15.1 (1.0)	12.0 (2.2)	19.1 (1.1)	175.2 (11.7)	35.2 (2.1)	27.0 (1.1)	36.8 (2.3)

Differences from control are indicated by lower case superscripts, differences from the preceding value (significance tests made only for Experiment 3) by upper case superscripts:
^{a,A} $P<0.1$; ^{b,B} $P<0.05$; ^{c} $P<0.01$; ^{d} $P<0.001$; 2-sided paired t-test

curves and the absence of high-amplitude and low-frequency periods that would reflect SWS. Motor activity was higher in the dark-phase than in the light-phase. In the EEG-sample shown in Figure 5 the animal was mostly quiet except for brief periods of locomotion which are reflected in a decrease of EEG-amplitude. Thus a slight increase in amplitude could occur for a few seconds as the animal rode passively in the slowly rotating cylinder. However, the low-frequency part of the EEG-spectrum remained at a low power-level during such periods (Fig. 5). In summary the SD-procedure was effective in preventing the deeper levels of NREM-sleep and probably also all REM-sleep.

Sleep-Deprivation Terminating at Light-Onset (Experiment 1)

In Experiment 1 the 12-h and 24-h SD periods terminated at light-onset, the beginning of the rat's daily rest-phase. On the day following the 12-h SD, only total sleep in the light-phase was significantly enhanced (Table 1). Rather surprisingly, NREM-sleep in the light-phase was reduced after 24-h SD, while both REM-sleep and the ratio REM/TS were increased. A tendency for an increase of the latter parameters was still evident in the dark-phase of the second day following SD (day 7). The marked REM-rebound after 24-h SD occurred as a very regular

Table 2. Episode duration (min) and episode frequency (per hour) of total sleep (TS), NREM-sleep and REM-sleep for the control day (Contr.), the day after 12-h sleep-deprivation (SD) and the day after 24-h SD (Experiment 1). Data represent arithmetic mean values (SEM in parenthesis), and are indicated for successive 3-h periods of the light-phase and for 11.5 h of the dark-phase. As in previous experiments (Borbély and Neuhaus, 1978a, b) episodes and interruptions lasting less than 30 s were disregarded

		Light				Dark
	Hours	1–3	4–6	7–9	10–12	13–23.5
Episode duration (min)						
TS	Contr.	9.2 (1.0)	8.0 (1.3)	9.0 (2.0)	7.5 (1.2)	3.5 (0.3)
	after 12 h SD	11.8[b] (0.6)	13.4[b] (2.2)	8.1 (1.0)	10.5 (1.8)	3.3 (0.6)
	after 24 h SD	18.5[b] (2.7)	12.5[b] (1.4)	18.0[a] (3.1)	9.5[a] (0.9)	4.8[b] (0.7)
NREM	Contr.	5.3 (0.5)	4.3 (0.4)	4.2 (0.5)	3.5 (0.3)	2.9 (0.2)
	after 12 h SD	6.5[a] (0.4)	5.5 (0.3)	4.0 (0.4)	4.0 (0.5)	2.5 (0.3)
	after 24 h SD	4.0 (0.3)	4.6 (0.3)	5.0 (0.3)	3.8 (0.2)	3.2[b] (0.3)
REM	Contr.	2.0 (0.1)	2.0 (0.1)	2.2 (0.2)	2.0 (0.7)	1.6 (0.1)
	after 12 h SD	2.2 (0.1)	2.7 (0.3)	2.3 (0.1)	2.2 (0.2)	1.6 (0.1)
	after 24 h SD	2.8 (0.4)	2.8 (0.2)	2.7[a] (0.2)	2.5 (0.2)	1.8 (0.1)
Episode frequency (per hour)						
TS	Contr.	5.2 (0.7)	6.1 (0.8)	6.1 (1.2)	5.8 (0.9)	2.9 (0.7)
	after 12 h SD	4.1 (0.3)	3.8 (0.6)	5.0 (0.9)	4.9 (0.6)	3.7 (0.5)
	after 24 h SD	2.9[b] (0.4)	3.3[b] (0.4)	2.7[b] (0.3)	4.7 (0.4)	3.2 (0.2)
NREM	Contr.	7.4 (0.6)	8.7 (0.7)	8.6 (1.0)	8.4 (0.7)	3.0 (0.7)
	after 12 h SD	6.8 (0.5)	6.8[b] (0.3)	7.9 (0.6)	8.8 (0.7)	4.1 (0.4)
	after 24 h SD	8.4 (0.6)	6.8 (0.5)	6.5 (0.4)	8.0 (0.5)	3.9 (0.4)
REM	Contr.	3.6 (0.5)	4.6 (0.3)	4.7 (0.4)	5.2 (0.4)	0.7 (0.2)
	after 12 h SD	3.4 (0.2)	3.9[a] (0.2)	4.1 (0.3)	6.0 (0.5)	0.9 (0.2)
	after 24 h SD	6.6[b] (0.5)	5.3 (0.3)	5.0 (0.3)	5.3 (0.6)	1.6[b] (0.3)

Differences from control:
[a] $P<0.1$; [b] $P<0.05$; 2-sided paired t-test

alternation between REM and NREM-sleep which is reflected by the oscillation of the EEG-amplitude and ZCR curve (Fig. 4). The changes of episode duration and episode frequency of TS, NREM-sleep and REM-sleep are indicated in Table 2. The decreasing trend of the episode duration of TS and NREM-sleep during the light-phase in the control day has been described in detail previously (Borbély and Neuhaus, 1978a, b). The 24-h SD resulted in an increase in TS-episode duration and a decrease in TS-episode frequency in the light-phase, changes that were less prominent and shorter lasting after 12-h SD. The episode duration of TS and NREM-sleep was still prolonged in the dark-phase following the 24-h SD. The longer deprivation period enhanced both the duration and frequency of REM-sleep episodes, although significant changes were seen only for some of the values.

The EEG-amplitude of NREM-sleep in the light-phase was significantly higher than the control level after both 12-h SD and 24-h SD (Fig. 4; Table 3). The amplitude increase was most prominent in the first 3-h period of recovery sleep, and then gradually declined (Table 3: right columns). There was no significant difference in the amplitude increase between the 12-h SD and the 24-h SD condition. The amplitude of REM-sleep remained unchanged on the day following SD.

Differences between the two deprivation conditions were clearly present, however, for the ZCR-bands of NREM-sleep. As can be seen on the sample plots shown in Figure 4, the ZCR-values of NREM-sleep were moderately reduced after 12-h SD, and markedly decreased for several hours after 24-h SD. It is evident also from the hourly mean values of the ZCR-bands (Fig. 6) that the SWS-fraction of NREM-sleep (< 40 ZCR/10 s; black area) increased in relation to the length of preceding SD. The EEG-spectra show that the low ZCR-values after 24-h SD resulted from the marked predominance of slow waves in the delta band during prolonged episodes of SWS (Fig. 5: bottom records). In terms of a quantitative comparison, the numbers of 10-s periods with less than 40 and less than 50 ZCR/10 s were both significantly different between the light phase of day 1 and day 3 as well as between day 3 and day 6 ($P<0.05$; 2-sided paired t-test).

On the second post-deprivation day, REM-sleep in the dark phase was still significantly enhanced after 24-h SD, and motor activity was increased after both 12-h SD and 24-h SD (Table 1). The EEG-amplitudes were at the control level 2 days after the 12-h SD. However, a striking and statistically significant reduction in amplitude by approximately 10% was observed for both NREM-sleep and REM-sleep

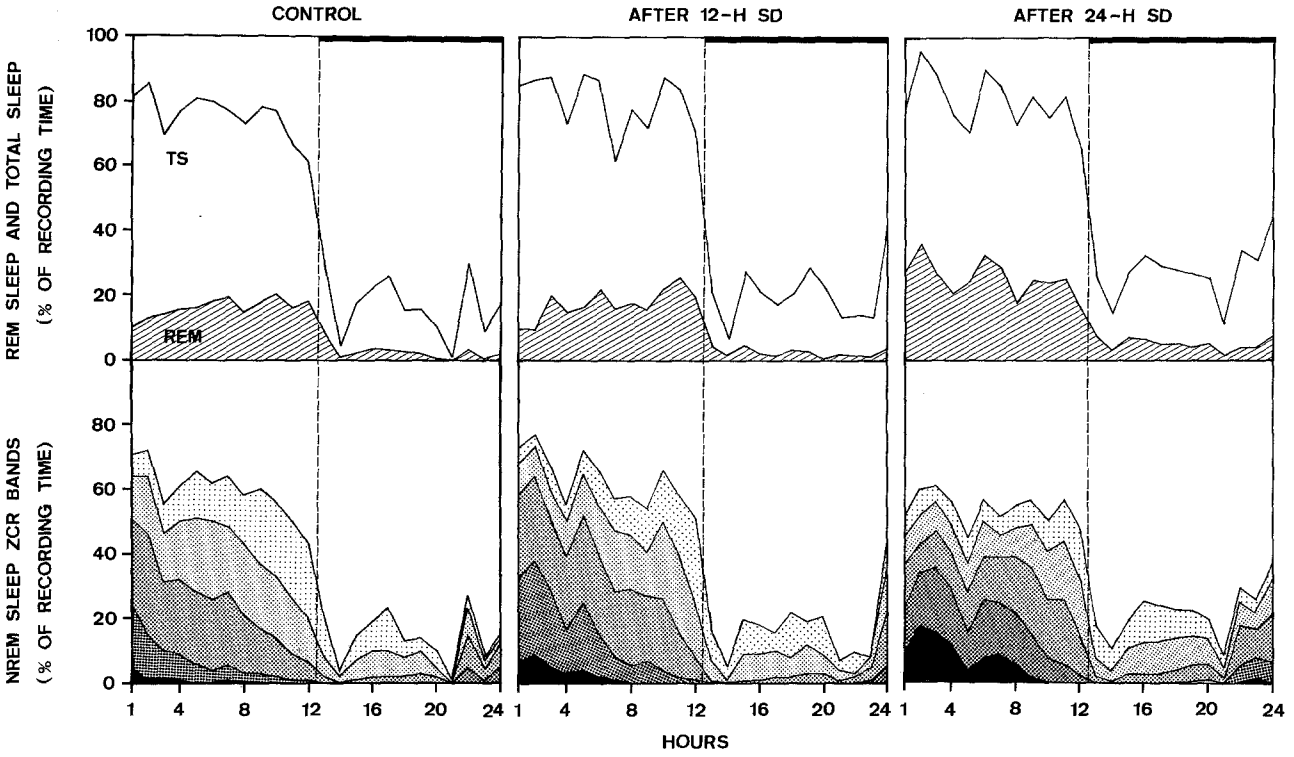

Fig. 6. Daily distribution of total sleep (TS) and REM-sleep (upper records), and of NREM-sleep with zero-crossing bands (lower records) during control day (left), the day after 12-h sleep deprivation (SD) (middle); and the day after 24-h SD (right). The curves represent mean hourly values expressed as the percentage of recording time. Areas of increasing darkness indicate decreasing zero-crossing bands as in Fig. 2. Dark bar on top abscissa and interrupted vertical line delimit the dark-phase

2 days after 24-h SD (Table 3). Since we did not seek to obtain evidence for a normalization of the amplitudes on the subsequent days, we hesitate to interpret these findings in terms of SD-aftereffects. Food and water consumption did not exhibit conspicuous variations during Experiment 1 apart from a decrease in food intake from day 6 to 7 ($P<0.01$; 2-sided paired t-test).

Sleep-Deprivation Terminating at Dark-Onset (Experiment 2)

In Experiment 2 the 24-h SD period terminated at dark-onset, the beginning of the rat's daily activity-phase. Figure 7 shows the records of an individual animal and Figure 8 illustrates the daily distribution of the hourly mean values for sleep and the EEG-parameters. A sleep period with a high percentage of REM-sleep was present during the first part of the dark-phase. However, soon the typical bouts of high motor activity with intervening rest periods dominated the picture (Fig. 7). Total sleep, REM-sleep and the REM/TS ratio were significantly increased and food intake reduced in the dark-phase following 24-h SD (Table 1). A moderate, but statistically significant increase of REM-sleep and REM/TS was still present in the light-phase of day 3.

The EEG-amplitude of NREM-sleep showed an average increase by 6.4% in the dark phase following SD (day 3) which did not reach the level of statistical significance (Table 3). However, the increase in the first 3-h period of the dark-phase by 7.2% was significant at the 0.01 level (2-sided paired t-test) and is also clearly evident in Figure 7. As in Experiment 1, the amplitudes of NREM-sleep and REM-sleep tended to be below the control level on the second post-deprivation day.

In comparison to the dark-phase of the control day, SWS was significantly increased after SD (<40 ZCR/10 s: $P<0.05$; 40–50 ZCR/10 s: $P<0.01$; 2-sided paired t-test). However, in contrast to Experiment 1, the SWS-rebound occurred in 2 stages, the second stage being situated at the beginning of the light-phase (Fig. 8).

Excess REM-Sleep and Excess Slow Wave Sleep After Sleep-Deprivation

Both REM-sleep and SWS (defined as NREM-sleep with <40 ZCR/10 s) were increased after 24-h SD

Table 3. EEG-amplitudes of NREM-sleep and REM-sleep in the light-phase (L) and dark-phase (D). The data represent arithmetic mean values (SEM in parenthesis). The NREM-sleep amplitudes of the L-phase for Experiment 1 are shown also for successive 3-h periods (right columns). In the top 3 rows the data are expressed as the percentage of the daily mean amplitude of NREM-sleep on day 1. The other data are expressed as the percentage of the corresponding control value on day 1

Experiment	Day	NREM		REM		NREM (light-phase)			
		L	D	L	D	1–3	4–6	7–9	10–12 (h)
1	1	98.6 (0.5)	106.4 (2.1)	76.4 (2.6)	75.8 (2.4)	106.0 (1.4)	97.2 (0.8)	95.4 (1.3)	94.0 (1.2)
2	1	97.6 (0.7)	109.4 (3.6)	64.9 (3.6)	65.2 (3.8)				
3	1	99.2 (0.8)	103.7 (3.0)	72.2 (4.0)	69.8 (4.8)				
1	1	100	100	100	100	100	100	100	100
	2	12-h sleep-deprivation							
	3	110.0^{c} (1.8)	101.5 (3.1)	103.0 (2.4)	101.6 (2.2)	112.7^{c} (2.2)	110.6^{c} (1.9)	106.5^{b} (1.7)	103.6 (2.8)
	4	99.6 (2.2)	98.7 (2.4)	100.4 (3.0)	99.2 (2.4)	98.9 (1.7)	101.1 (2.8)	99.2 (2.9)	99.9 (2.7)
	5	24-h sleep-deprivation							
	6	107.8^{a} (3.2)	95.7 (3.3)	100.0 (2.6)	97.1 (2.4)	109.3^{b} (3.0)	110.6^{b} (3.5)	108.0^{a} (3.7)	103.9 (3.4)
	7	90.1^{c} (2.1)	86.8^{c} (2.5)	92.6^{b} (2.3)	88.8^{c} (1.9)	89.1^{c} (2.1)	90.5^{b} (2.9)	90.5^{b} (2.4)	90.8^{b} (3.1)
3	1	100	100	100	100				
	2	12-h sleep-deprivation: usual rot. rate							
	3	106.4^{d} (0.5)	102.7 (2.7)	100.5 (0.9)	104.8 (3.0)				
	4	12-h sleep-derprivation: double rot. rate							
	5	102.6 (2.8)	98.2^{B} (3.3)	98.7 (1.8)	102.1 (4.5)				
		D	L	D	L				
2	1	100	100	100	100				
	2	24-h sleep-deprivation							
	3	106.4 (4.2)	96.6 (3.3)	100.6 (1.2)	96.2 (1.3)				
	4	91.9 (3.2)	92.0 (4.3)	92.4^{a} (5.3)	88.9^{a} (3.1)				

Differences from control (day 1) are indicated by lower case superscript, differences from preceding value (significance tests made only for Experiment 3) by upper case superscript:
^{a} $P<0.01$; ^{b,B} $P<0.05$; ^{c} $P<0.01$; ^{d} $P<0.001$; 2-side paired t-test

in Experiment 1 and 2. The amount of excess REM-sleep on the post-deprivation day was similar in both experiments (Exp. 1: 166.3% (8.2 SEM) of control; Exp. 2: 168.9% (11.5 SEM)). Also the timecourse of the REM-rebound was surprisingly similar under the two conditions, with approximately 80% of the excess REM-sleep occurring within the first 12-h period (Fig. 9). In contrast to REM-sleep, the timecourse of SWS-rebound was different between the two experimental schedules. While in Experiment 1, excess SWS occurred almost exclusively within the first 12-h light-phase, SWS-rebound took place in two stages in Experiment 2: The first stage occurred predominantly within the first 3 h of the dark-phase, and the

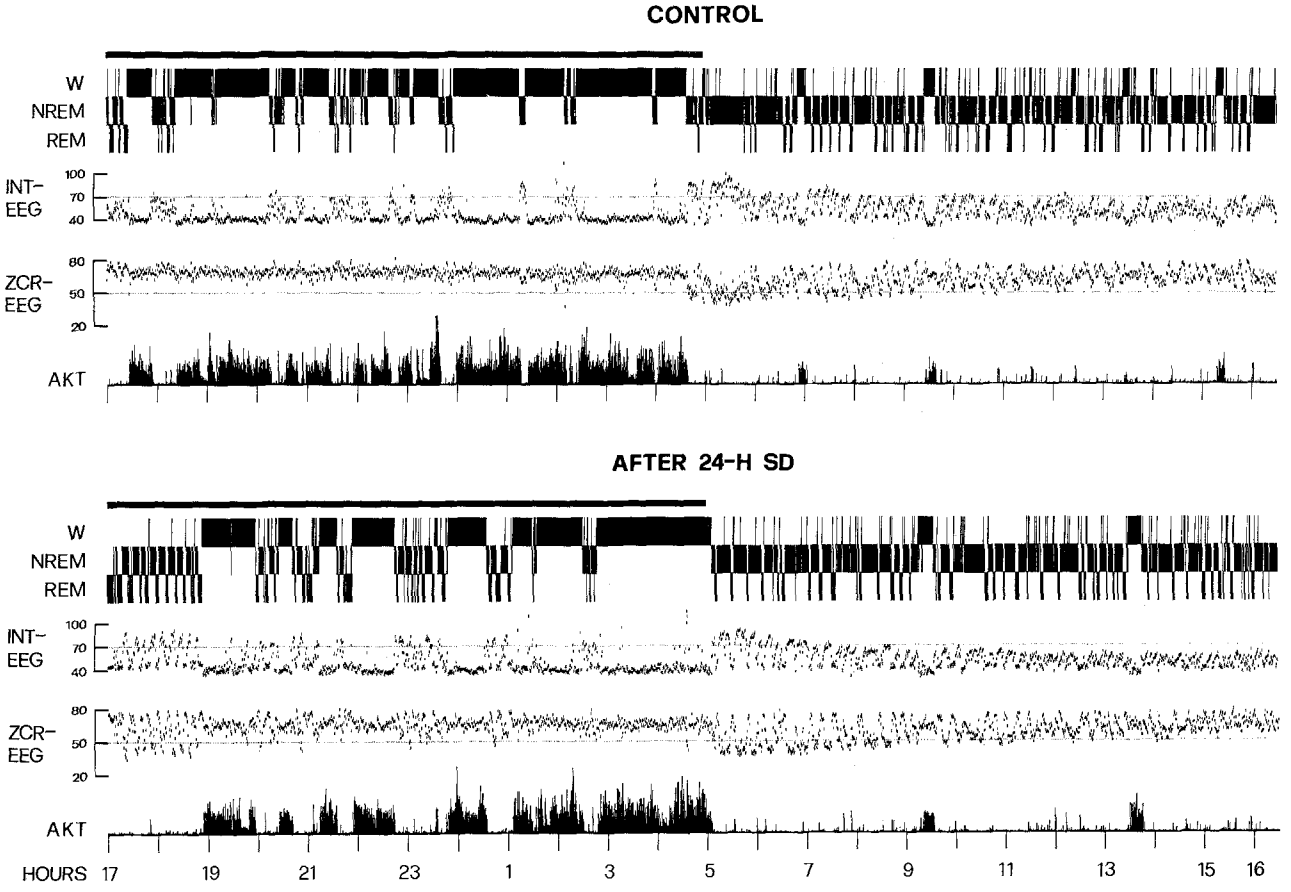

Fig. 7. Vigilance states, EEG-parameters and motor activity of a rat plotted for the control day (upper records) and the day after 24-h sleep-deprivation (lower records). See legend of Fig. 4 for abbreviations and details. Note that in contrast to Fig. 4 recovery sleep starts at dark-onset. (Animal 13)

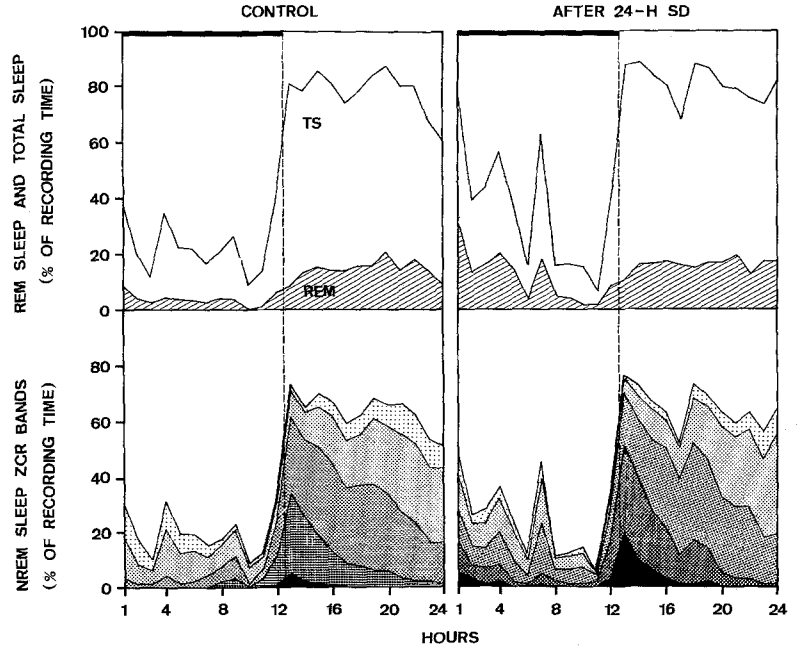

Fig. 8. Daily distribution of total sleep (TS) and REM-sleep (upper records), and of NREM-sleep with zero-crossing bands (lower records) during control day (left) and after 24-h sleep-deprivation (right). See legends of Figs. 2 and 6 for details. Note that in contrast to Fig. 6 recovery sleep starts at dark-onset

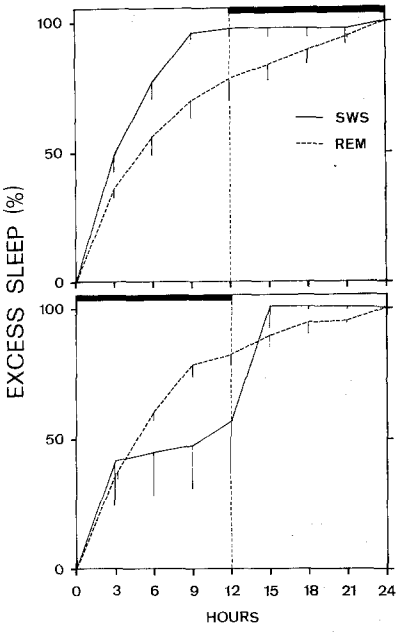

Fig. 9. Cumulative curves of excess slow wave sleep (SWS = NREM-sleep with less than 40 ZCR/10 s; solid line) and excess REM-sleep (interrupted line) during the day after 24-h sleep-deprivation (SD) of Experiment 1 (upper record) and Experiment 2 (lower record). Dark bar below top abscissa and interrupted vertical line delimit the dark-phase. The data represent arithmetic mean values with SEM for successive 3-h periods. Excess sleep is defined as the difference between the value on the day after SD and the control day, and is plotted as 100% irrespective of the absolute amount (see text for details)

second stage in the first 3 h of the light-phase, 12 h after the end of SD. The rising tendency of the SWS-curve before light-onset (Fig. 9) is due to the fact that the daily rest-phase began before the end of the dark-phase in some animals (see Fig. 7: control).

Since SWS was defined in terms of the 10-s periods satisfying the zero-crossing criterion, and since the amount of SWS so defined represented a small fraction of total sleep and showed a considerable variability between animals, a quantitative comparison of SWS between the two experiments should be interpreted with caution. The average control level of SWS was comparable in Experiment 1 and 2 (Exp. 1: 37.2 periods/day (SEM 15.5); Exp. 2: 40.4 periods/day (SEM 7.7)). The average increase on the day after 24-h SD was to 824% of control in Experiment 1, and to 468% in Experiment 2 (difference not significant). On the second day after SD, the average level of SWS was at 80% of control in Experiment 1, but still at 238% in Experiment 2 ($P<0.1$; 2-sided paired t-test). The excess SWS which was present in 3 out of 4 rats on the second post-deprivation day of Experiment 2, occurred in the first part of the light-phase, approximately 36 h after SD. This observation indicates that when recovery from SD starts in the dark-phase, the rebound of SWS takes place not only in the two stages illustrated in Figure 9, but may be succeeded by an even more delayed stage.

Sleep-Deprivation Performed with Different Cylinder Rotation Rates (Experiment 3)

To evaluate the role of forced locomotor activity, 12-h SD was performed with two different cylinder rotation rates. The effects of the two 12-h SD schedules were comparable to those described for 12-h SD in Experiment 1 (Table 1). Figure 10 shows the mean hourly values for TS, REM-sleep and NREM-sleep with the ZCR-bands. Only minor differences were observed between the two schedules. They were mainly due to the attenuation of the light-dark differences when SD was performed with the double rotation rate (Table 1). The EEG-amplitudes in the light-phase were above the baseline level after both SD-procedures, yet significant differences were present only on day 3 (Table 3). SWS was also increased after both SD-schedules, but only the value for the 40–50 ZCR-band on day 3 was significantly above control ($P<0.01$; 2-sided paired t-test). So, if anything, the changes were more prominent when SD was performed at the usual rotation rate. However, the relatively close succession of the two SD-experiments prevents any conclusion on this point.

Discussion

Slow Wave Sleep on Control Days and After Sleep Deprivation

The present study was prompted by the search for electrophysiological correlates of recovery during sleep. It is reasonable to assume that a parameter reflecting a recovery process should exhibit a progressive change as sleep proceeds. Progressive changes were manifested during the daily rest-phase of the present experiments mainly by the prominent decreasing trend of the EEG-amplitude and the marked increasing trend of the dominant EEG-frequency (as reflected by the ZCR-values) of NREM-sleep. These observations confirm and extend the results of Rosenberg et al. (1976) who described the daily variation of slow wave sleep (SWS) in the rat[1]. The predomi-

[1] These authors pointed out that the use of the term 'slow wave sleep' as a synonym of NREM-sleep is incorrect. We fully support this statement, since in the present study the high-amplitude signal of NREM-sleep exhibited a wide range of frequency distributions. In analogy to the terminology used for human sleep, the term 'slow wave sleep' should be reserved for NREM-sleep with a low-frequency EEG-signal

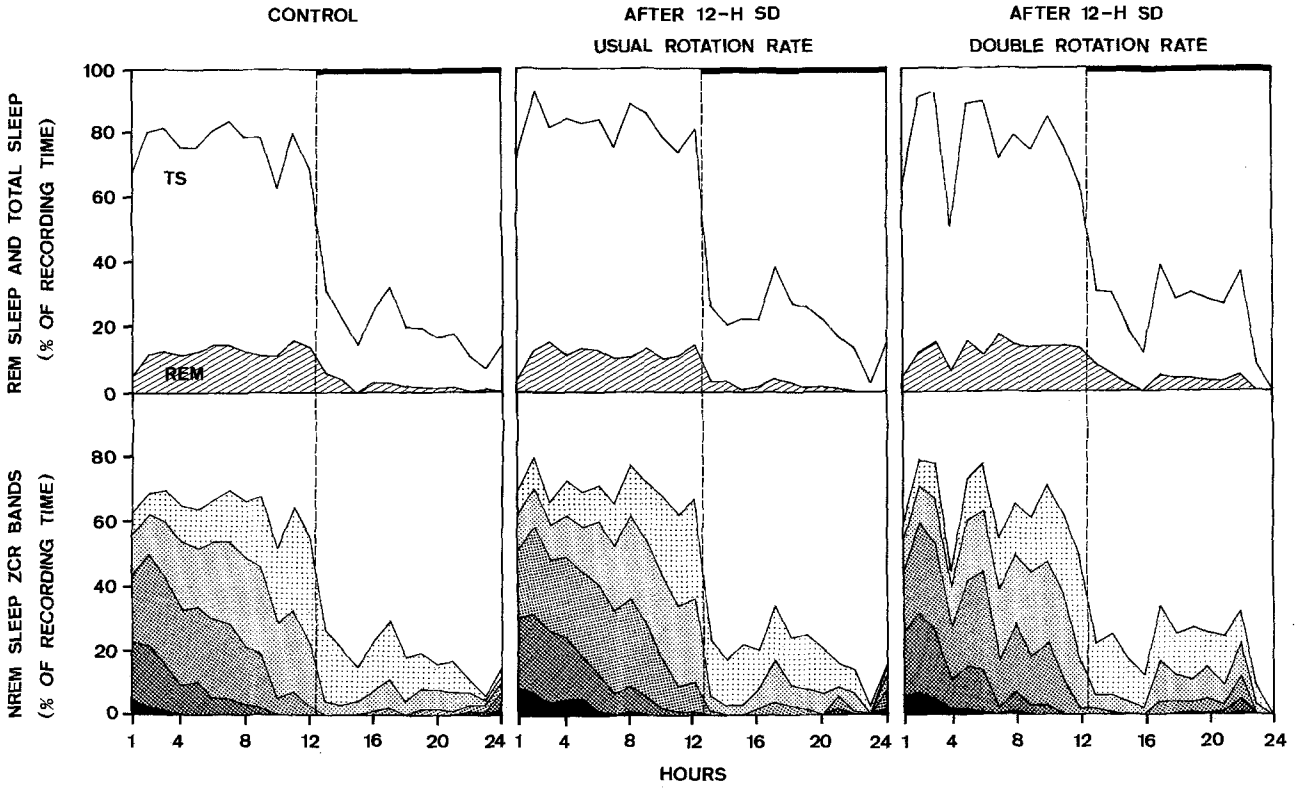

Fig. 10. Daily distribution of total sleep (TS) and REM-sleep (upper records), and of NREM-sleep with zero-crossing bands (lower records) during control day (left); after 12-h sleep-deprivation (SD) performed at the usual cylinder rotation rate (middle); and with the double rotation rate (right). See legends of Figs. 2 and 6 for details

nance of SWS in the early part of the daily sleep-phase which was conspicuous in the present study, is well documented for sleep in man (e.g. Webb and Agnew, 1971) and monkey (Reite et al., 1965; Crowley et al., 1972), and also the decreasing trend of the EEG-amplitude during sleep has been reported for human experiments (Sinha et al., 1972; Church et al., 1977; Smith et al., 1977). The progressive shortening of the NREM-sleep episodes in the rat has been described previously and related to similar changes in man (Borbély and Neuhaus, 1978a). Thus various NREM-sleep parameters exhibit in different species corresponding trends during the daily sleep-phase.

If NREM-sleep parameters do represent correlates of a recovery process, their trend in the sleep-phase should be accentuated after sleep-deprivation. In the present experiments, a major consequence of SD was the increase in the amount of SWS as defined by the lowest zero-crossing band. The extent of SWS-increase was a function of the duration of SD. The enhancement of SWS by SD is well known for man (Berger and Oswald, 1962, for the first detailed study), and has been described also for the cat (Ursin, 1971) and dog (Takahashi et al., 1978). The augmentation of the EEG-amplitude of SWS has been observed in the sleep-deprived rabbit (Pappenheimer et al., 1975). Thus both the amplitude and the dominant frequency of the EEG during NREM-sleep may constitute electrophysiological correlates of a recovery process. However, the two EEG-measures may not represent two facets of the same process. Although an inverse relationship between EEG-amplitude and EEG-frequency was usually present on control days (Fig. 2), a partial independence of the two EEG-parameters became evident after SD. Thus, unlike EEG-frequency, the change of EEG-amplitude in NREM-sleep did not depend on the duration of the deprivation period. Furthermore, in Experiment 2, an increase in amplitude was present only immediately after SD, whereas the decrease in frequency was seen at both an immediate and a delayed stage. Also in human sleep, the usual inverse relationship between EEG-amplitude and EEG-frequency is not invariantly present, since the amplitude measure of SWS exhibits a decreasing trend during sleep, while the dominant delta-wave frequency is lowered both within and between successive SWS-periods (Church et al., 1977; Smith et al., 1977). Thus although both EEG-parameters exhibit comparable trends on control days and after SD, they may still reflect different aspects of recovery.

Since physical exercise has been reported to en-

hance sleep in the rat (Matsumoto et al., 1968) and cat (Hobson, 1968), and similar, though controversial claims have been made for man (Shapiro et al., 1975; Griffin and Trinder, 1978; Walker et al., 1978), we attempted to evaluate the influence of this factor by studying the effects of SD performed by two different cylinder rotation rates. Within the limits of the experimental design, the increased forced locomotion did not enhance the SD-effects on sleep and on EEG-parameters. Exercise does not seem therefore to constitute a major factor in the present study. This conclusion is supported by previous SD-experiments in animals (e.g. Takahashi et al., 1978) and man in which the enhancement of SWS was observed in the absence of forced locomotion.

Compensation of Slow Wave Sleep and REM-Sleep: The Question of Priority

It has been argued that SWS may be more intimately related to recovery than REM-sleep, since in various human SD-studies a SWS-rebound occurred prior to an increase in REM-sleep (Berger and Oswald, 1962; Williams et al., 1964; Moses et al., 1975; Nakazawa et al., 1978). This assumption is further supported by the observation that during a prolonged regime with restricted sleep, SWS was enhanced, whereas REM-sleep remained below the baseline level (Webb and Agnew, 1974). Also some of the present results are consistent with the notion of a high priority of SWS: Thus an immediate SWS-rebound was seen under all SD-schedules, including the 12-h SD condition which did not significantly affect REM-sleep. On the other hand the prominent rebound of REM-sleep after 24-h SD occurred concomitantly with the SWS-rebound, the excess REM-sleep being even less affected by circadian influences than the excess SWS (Fig. 9). However, also the data from human studies are not consistently in favour of a SWS-priority over REM-sleep. Thus a concomitant rebound of SWS and REM-sleep has been reported for human subjects recovering from a severe SD (Gulevich et al., 1966; Kales et al., 1970), and a circadian influence on SWS has been equally noticed (Webb and Agnew, 1971; Moses et al., 1975), although the recovery from SD at different phases of the circadian rhythm has not been investigated in man. As will be argued in the next section, the apparent contradictions with respect to the concept of sleep state priority may be resolved, if the different regulatory mechanisms for NREM-sleep and REM-sleep are taken into account.

Is there a difference between the amount of excess SWS and excess REM-sleep after SD? In the present experiments only approximately 70% of the lost REM-sleep was recovered on the first recovery day after 24-h SD, whereas an over-compensation of the amount of lost SWS (NREM-sleep with <40 ZCR/10-s) was observed. However, a quantitative comparison between REM-sleep and SWS is of questionable validity, since SWS was defined on the basis of an arbitrary threshold. Moreover, the crude estimate of EEG-frequency by the zero-crossing method did not allow to specify in detail the EEG-parameters that were affected by SD (e.g. delta wave frequency, number of delta waves, delta wave density). A period and amplitude analysis as developed by Feinberg and colleagues (1978) may provide more insight into this problem.

The main part of sleep compensation occurred on the first recovery day. However, altered levels of REM-sleep and motor activity were still evident on the second recovery day (Experiment 1), and indications for a delayed enhancement of SWS (Experiment 2) were equally observed. It has been repeatedly reported for human studies that the increase of SWS (Williams et al., 1964; Kales et al., 1970; Nakazawa et al., 1978) and REM-sleep (Berger and Oswald, 1962; Williams et al., 1964) persists longer than the first recovery night, but corresponding observations in animals were lacking. The long-term effects of SD deserve a careful study, since they may throw light on the nature and 'kinetics' of the hypothetical recovery process(es) subserved by sleep.

Sleep in the Framework of the Circadian Rest-Activity Rhythm

The circadian rest-activity rhythm is a major determinant of behavior in the rat as well as in many other animals (see Borbély, 1978, for a review). It precedes the sleep-waking rhythm on the evolutionary scale, since it is present in lower organisms in which sleep cannot be identified by the commonly used electrographic criteria. The circadian rest-activity rhythm is usually synchronized to the light-dark cycle, and thereby facilitates the adaptation of the organism to its environment. However, the rigid control of the behavioral pattern by circadian oscillators may have also negative consequences, since the rest periods occur at predetermined daily intervals, and not in relation to the requirements of the life situation. The emergence of sleep may constitute therefore a partial liberation from the limitation imposed by the circadian program, since sleep may enable a recovery process according to the momentary needs of the animal. However, the adaptive value of sleep may consist not so much in its more flexible timing than in its intensity dimension for which the predominant EEG-frequency in NREM-sleep seems to be an indicator. Thus recovery might be particularly efficient during

SWS, the 'deepest' (or highest intensity) part of NREM-sleep which appears relatively late in ontogeny (Jouvet-Mounier et al., 1969). In contrast to NREM-sleep, REM-sleep lacks an obvious intensity dimension. This statement is of considerable heuristic value for interpreting various experimental results, but it is not meant to imply that NREM-sleep and REM-sleep subserve identical functions. The functional significance of NREM-sleep 'intensity' is apparent from the present study, since after 24-h SD an increased amount of SWS was seen, even though the time devoted to NREM-sleep was reduced (Experiment 1, Table 1). On the other hand, REM-sleep rebound occurred as an increase in REM-sleep time which entailed by necessity a curtailment of the time available for other behavioral activities. Since a REM-deficit has to be 'repaid' in the hard currency of time, it is plausible that REM-sleep should not be regulated around its usual daily level, but rather around a level which is well below baseline. This would explain why the relatively mild loss of REM-sleep during the 12-h SD did not result in a REM-sleep rebound, whereas the more severe deficit incurred during 24-h SD gave rise to a marked increase. These arguments are consistent with the proposition of a facultative and an obligate REM-sleep quota by Parmeggiani and Rabini (1970). In contrast to REM-sleep, NREM-sleep can be easily regulated around its usual baseline level, an assumption that would allow to interpret the daily maximum of SWS at the beginning of the daily sleep phase as well as the SWS-increase after SD as a graded compensatory response. Moreover, the two-stage rebound of SWS in Experiment 2 indicates that the compensation of NREM-sleep may be delayed and thus adapted to the optimal circadian phase.

Even if sleep is viewed as a means for escaping the dictate of the circadian rest-activity rhythm, this escape is possible only within narrow limits and for short time periods. In most animals and in man, the major portion of sleep occurs within a well-defined circadian phase. In the rat, sleep is 'gated' by the sudden end of the activity phase when 'sleep pressure' is released and becomes manifest by long NREM-sleep episodes with a high proportion of SWS. However, it was apparent from the brief and infrequent episodes of NREM-sleep in the dark-phase that the propensity for a high-amplitude and low-frequency NREM-sleep increases *gradually* as the activity phase proceeds and the duration of waking increases (Fig. 2, 3). Human subjects taking naps at different periods of the day exhibited an analogous rise in the amount of SWS as the period of the preceding wakefulness increased (Webb et al., 1966, Karacan et al., 1970).

As a general conclusion, we wish to emphasize the usefulness of discriminating the changes occurring *within* NREM-sleep in animal experiments. The prominent daily trends of the EEG-frequency distribution and various effects of sleep deprivation, would have passed largely unnoticed, had the analysis been limited to the customary crude discrimination of 3 vigilance states. The more refined analysis of the sleep state based on the EEG-signal has substantially contributed to the recognition of correspondences between sleep in the rat and sleep in other mammals. The present results bear witness to the considerable invariance of sleep structure across species, a situation that may facilitate the search for functional processes subserved by sleep.

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