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Sleep EEG spectral analysis in a diurnal rodent: *Eutamias sibiricus*

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Summary. 1. Sleep was studied in the diurnal rodent *Eutamias sibiricus*, chronically implanted with EEG and EMG electrodes. Analysis of the distribution of wakefulness, nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep over the 24 h period (LD 12:12) showed that total sleep time was 27.5% of recording time during the 12 h light period and 74.4% during the 12 h dark period. Spectral analysis of the sleep EEG revealed a progressive decay in delta power density in NREM sleep during darkness. Power density of the higher frequencies increased at the end of darkness. Power density of the higher frequencies decreased and that of the lower frequencies increased during light.

2. Analysis of the distribution of vigilance states under three different photoperiods (LD 18:6; 12:12; 6:18) revealed that changes in daylength mainly resulted in a redistribution of sleep and wakefulness over light and darkness. Under long days the percentage of sleep during light was enhanced. The time course of delta power density in NREM sleep was characterized by a long rising part and a short falling part under long days, while a reversed picture emerged under short days. As a consequence, the power density during light was relatively high under long days.

3. After 24 h sleep deprivation by forced activity, no significant changes in the percentage of wakefulness and NREM were observed, whereas REM sleep was slightly enhanced. EEG power density, however, was significantly increased by ca. 50% in the 1.25–10.0 Hz range in the first 3 h of recovery sleep. This increase gradually decayed over the recovery night.

4. The same 24 h sleep deprivation technique led to a ca. 25% increase in oxygen consumption during recovery nights. While the results of the EEG spectral analysis are compatible with the hypothesis that delta power density reflects the 'intensity' of NREM sleep as enhanced by prior wakefulness and reduced by prior sleep, such enhanced sleep depth after sleep deprivation is not associated with reduced energy expenditure as might be anticipated by some energy conservation hypotheses on sleep function.

Introduction

Much of our knowledge on sleep regulation in mammals is based on just a few species. Apart from human subjects, only rats have been used extensively in laboratory studies (Campbell and Tobler 1984). Comparative studies have focussed on the relations between ecological niche and sleep patterns, and the descriptions of the latter were often limited to the temporal distribution of sleep states or the total durations of NREM and REM sleep (cf. Zepelin and Rechtschaffen 1974). Regulatory processes underlying the sleep-wake cycle were not investigated in these comparative studies. Some information on such processes is available for nocturnal rodents, but in diurnal rodents they are virtually unexplored.

The main experimental tool for investigating sleep regulatory processes has been sleep deprivation. In the rat, sleep deprivation studies have revealed that both homeostatic and circadian pro-

Abbreviations: *EEG* electroencephalogram; *EMG* electromyogram; *MUA* multiple unit activity; *NREM* non-rapid eye movement; *REM* rapid eye movement; *SCN* suprachiasmatic nucleus; *SWA* slow wave activity; *TST* total sleep time; *W* wakefulness

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cesses are involved in sleep regulation. The presence of a homeostatic regulatory process can be inferred from the changes in the 'intensity' of NREM sleep induced by variations in the prior sleep-wake history. The intensity of NREM sleep can operationally be defined as the arousal threshold for external stimuli. The threshold for acoustic stimuli increases after sleep deprivation in rats (Frederickson and Rechtschaffen 1978). In cats the awakening threshold for electrical brain stimulation is higher during SWS-2 (the NREM stage with more than 50% delta waves per epoch) than during SWS-1 (Grahnstedt and Ursin 1980).

After sleep deprivation slow wave activity (SWA) is enhanced, and this change has been interpreted as an increase in NREM sleep intensity (Borbély and Neuhaus 1979; Friedman et al. 1979; Borbély et al. 1984). The effects of total sleep deprivation are not fully restricted to NREM sleep. An increase in the duration of REM sleep following sleep deprivation has been reported in the rat (Borbély and Neuhaus 1979), while in more recent studies it was shown that during REM sleep power densities in the theta frequencies are enhanced (Borbély et al. 1984; Tobler and Borbély 1986).

Circadian aspects of sleep regulation in the rat have been most directly demonstrated by so-called conflict experiments (Borbély and Neuhaus 1979; Trachsel et al. 1986). When sleep deprivation ends at the beginning or in the second half of the activity phase, recovery sleep is only of short duration.

These observations are qualitatively compatible with a recent model of human sleep regulation (Borbély 1982; Daan et al. 1984) in which the timing of sleep and wakefulness results from an interaction of an hourglass process coding for the duration of prior wakefulness and a circadian clock which allows the organism to keep track of environmental time. The hourglass process is thought to be reflected in the intensity of NREM sleep. Key properties of this model are that the circadian clock determines a preferred phase for sleep, while NREM sleep intensity is a monotonic rising function of the duration of prior wakefulness and a monotonic decreasing function of the duration of prior sleep which in itself is not directly modulated by the circadian pacemaker. In humans, EEG power density in the delta and theta frequencies have been shown to increase monotonically with increasing duration of prior wakefulness (Dijk et al. 1987a). During sleep, power density in these frequencies decreases (Borbély et al. 1981). That the time course of delta power density during sleep is indeed regulated by an hourglass process was shown by an experiment in which delta power density during the first part of sleep was reduced by acoustic stimuli (Dijk et al. 1987b). In the second part of sleep delta power density turned out to be enhanced relative to the comparable part of baseline sleep. This result indicates that the time course of delta power density during sleep is not determined by the time passed since sleep onset but by the power produced during the preceding part of the sleep episode.

Whether this model is at least qualitatively applicable in diurnal rodents has not been investigated. We have therefore undertaken a study of the sleep-wake cycle in the chipmunk, with particular emphasis on the homeostatic aspects of sleep-wake regulation. Sleep was analyzed both in terms of sleep stages and by means of spectral analysis, under baseline conditions, and after sleep deprivation. We further studied 24 h patterns of sleep under long and short days since these represent natural variations in the time available for nocturnal sleep. The functional significance of NREM sleep and changes in its intensity remain to be established. Ecological theories on sleep function have emphasized that metabolic rate is reduced during sleep (Berger 1984). If energy conservation is indeed the primary function of NREM sleep, it can be hypothesized that changes in NREM intensity are correlated with changes in metabolic rate. In humans O_2 consumption does reach the lowest values during the deepest NREM stages, i.e., stages 3+4 (Brebbia and Altschuler 1965). Likewise, low levels of mean cerebral metabolism are associated with NREM sleep in cats (Ramm and Frost 1986). To our knowledge no data are available on the effects of sleep deprivation on O_2 consumption in small rodents. We have therefore complemented our study of the effects of sleep deprivation on the EEG with the assessment of its effects on metabolic rate.

Eutamias sibiricus (the Siberian chipmunk or Burunduk) is a diurnal rodent living in the northern part of the Soviet Union, Siberia, the Far East, Northern China, and Japan. Some aspects of its circadian rest-activity cycle and sleep-wake cycle have been described (Pohl 1972, 1976, 1982). The circadian organization of these cycles are, as in the rat and hamster (Rusak and Zucker 1979), dependent on the suprachiasmatic nuclei (SCN) (Sato and Kawamura 1984a). However, the chipmunk differs from the rat since in the rat the rest phase coincides with the time of highest SCN multiple unit activity (MUA) (Inouye and Kawamura 1979), whereas in the chipmunk MUA is highest during the active phase (Sato and Kawamura 1984b). Hence, the time course of MUA of the

SCN relative to the light-dark cycle is similar for the nocturnal rat and the diurnal chipmunk. If SWA is only determined by prior sleep and wakefulness and does not depend on circadian phase, SWA should decrease progressively during the major rest phase also in the chipmunk. Furthermore, SWA should be enhanced after sleep deprivation.

Methods

Adult, wild caught animals of unknown age were obtained directly from Korea via a commercial pet shop. Animals used in the experiment were all sexually mature males (body weight: 89–137 g). In the laboratory they were kept in groups in large cages. Ambient temperature was 23° C. Under these conditions chipmunks do not hibernate (Sato and Kawamura 1984). Food and water were given ad libitum. The diet consisted of various weeds, cedar nuts, lucerne, fruits, and rat chow (Muracon). Under anesthesia silver electrodes were implanted on the dura above the parietal cortex and above the cerebellum. A third electrode was attached to stainless steel screws placed near the frontal cortex, which served as a common ground. EMG electrodes (Plastic Products Company MS 303/71) were attached subcutaneously to the dorsal neck muscles.

After the operation the animals were individually housed in cages (54·38·41 cm) in which they had access to a running wheel (diameter 44 cm). At least 1 week of recovery was allowed. Some 48 h before an EEG recording the animals were connected to the recording leads to allow adaptation. Then, 16–24 h before the start of a recording the animal in its cage was transferred to a sound attenuated room. During the light phase the animals could be observed via a one-way screen. To prevent any interaction no other animals were present in the recording room.

The EEG and EMG were recorded on an Elema Schönander polygraph with a paperspeed of 5 or 10 $mm \cdot s^{-1}$. Filtersettings were 30 Hz, time constant 0.6 s for EEG and 700 Hz, time constant 0.015 s for EMG signals. For quantification of the EEG signal two methods were used. In the first and second experiments, the EEG was fed into nine parallel analogue bandpass filters (Burr-Brown; type UAF41). Center frequencies of these filters were 0.75, 1.5, 2.5, 3.5, 4.5, 6.0, 8.0, 10.0, and 13.5 Hz. The high and low pass 3 dB points of the filters were positioned symmetrically (on a linear scale) around the center frequency. The high and low 3 dB points of adjacent filters coincided. The output from each filter was squared and integrated over 10 s epochs. The EMG signal was also squared and integrated over 10 s epochs. According to Parcevals relation (Rabiner and Gold 1975, p. 36) the obtained values are proportional to signal power (for further discussion of the similarities between this method and fast Fourier transformation see Mendelson et al. 1980). An Apple computer stored the 10 s values on floppy disk. To allow synchronization between the paper recordings and power data, a time signal was recorded on the EEG paper. The EEG was visually scored per 10 s epochs. Three vigilance states were distinguished: wakefulness, NREM sleep, and REM sleep on the basis of EEG and EMG characteristics (see Results). The scores were also stored on floppy disk. Software written in Pascal enabled us to calculate power densities per frequency band and per vigilance state. Power spectra during wakefulness were not calculated since many artefacts due to movements were present during this state.

In the third experiment the EEG was recorded on analogue tape (Philips analog-7). The signal was played back into an A/D converter with a sampling rate of 64 Hz and stored on magnetic tape. Power densities between 0.25 and 15.0 Hz were calculated per 4 s epoch by means of a fast Fourier transformation on a PDP11/34 computer. Data were stored per 1 Hz bin per 4 s epochs. The paper recordings were scored per 30 s epoch and matched with the power spectra. Since this time resolution is rather limited, power spectra were calculated for NREM and REM sleep combined. Power densities during wakefulness were not analyzed.

In the first experiment eight animals were recorded for 24 h under LD 12:12. The EEG recording started at the beginning of the light period. In the second experiment the effects of different photoperiods (LD 6:18, 12:12, 18:6) on the distribution of the vigilance states and the time course of delta power density were studied in ten animals (eight of these animals were also used in Experiment 1). After a 24 h EEG recording under LD 12:12, the light period was extended by 6 h by advancing lights on for 3 h and delaying lights off for 3 h. After approximately 3.5 weeks 24 h EEG recordings were made. Next, the light period was recuded to 6 h by delaying lights on for 6 h and advancing lights off for 6 h. After approximately 8.5 weeks the final 24 h EEG recordings were made. All other conditions were identical to those in Experiment 1. In the third experiment the effects of 24 h forced activity on vigilance states and power spectra during the dark period were investigated in a group of seven animals. The light-dark schedule was LD 12:12. After recording of a baseline night the animals were placed in a slowly rotating drum (diameter 45 cm; 1 revolution per 19 s) at the end of the light period. Then, 24 h later (i.e. at the beginning of the dark period), the animals were returned to their home cage, and recovery sleep was recorded for 12 h. The animals had access to water and food throughout the experiment.

In the fourth experiment O_2 consumption during the night was measured in six animals, before and after 24 h sleep deprivation by forced activity. The animals were placed in a metabolic chamber (31·20·22 cm) of an open flow system to which the animals had been adapted during one night. The chamber was placed in a temperature-controlled cabinet (23° C). During the baseline night and the recovery night, the oxygen concentration of the dry outflowing gases was measured with a S3A applied electrochemistry oxygen analyzer and recorded continuously, except for 15 min every 2 h when O_2 of inflowing air was recorded. O_2 consumption was calculated according to Eqn. 2 of Hill (1972) and expressed at standard temperature and pressure (0° C, 1 atm). In four of the six animals, O_2 consumption was also measured during the second night after sleep deprivation.

Results

Experiment 1: 24-h EEG records

Vigilance states. On the basis of the EEG records and observations during the light phase three vigilance states were distinguished: Wakefulness (W), NREM sleep, and REM sleep. In Fig. 1 A examples of the EEG and EMG records characteristic of the three states are given. Figure 1 B shows the squared outputs from the filters and Fig. 1 C, the relative contribution of each filter to total power during the 10 s epoch. During wakefulness the EEG consisted of a low amplitude mixed frequency EEG and muscle tone was high. Occasionally delta

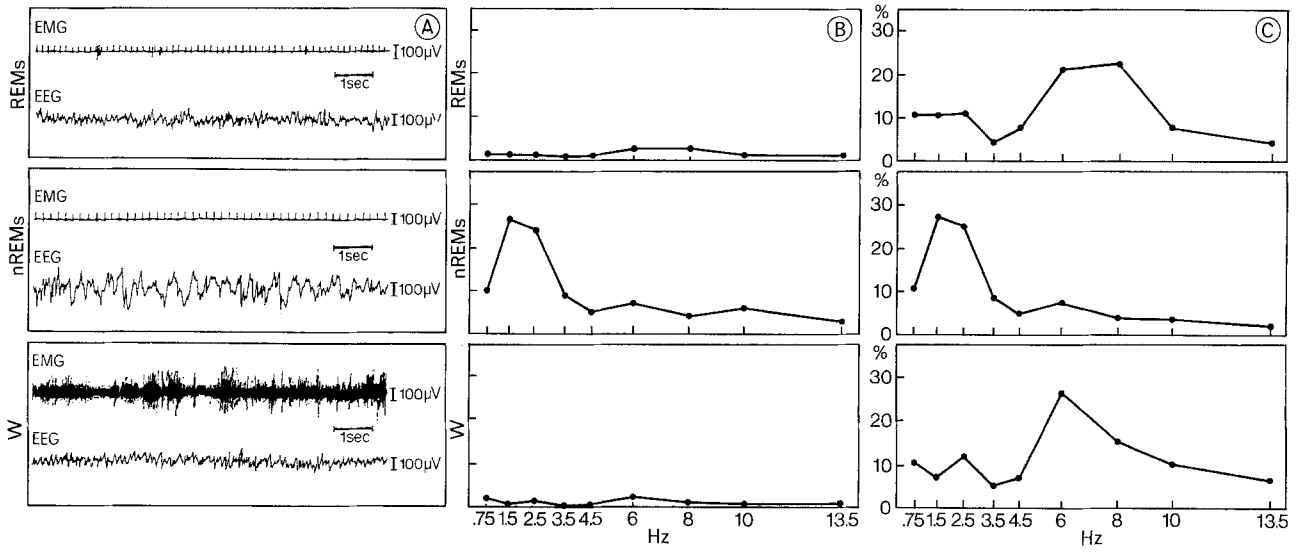

Fig. 1. EEG and EMG recordings of REM, NREM, and wakefulness (**A**). Absolute (**B**) and relative (**C**) power spectra for these epochs are also shown. Note the twitches and heart beat in the EMG signal. Absolute power densities are in arbitrary units

waves were observed when the animal was sitting with eyes half open and high muscle tone. These epochs were scored as W. During NREM sleep muscle tone was lower, and spindles and delta waves could be observed. During REM sleep a prominent theta rhythm was present in the absence of muscle tone. Twitches in the EMG could occasionally be observed.

During the 12 h light period the animals were awake for 72.5% ± 5.5% (sd) of recording time. Time in NREM sleep was 23.7% ± 4.1%, whereas 3.6% ± 3.0% of recording time was spent in REM sleep. During the 12 h darkness the data for the last 6 h were missing in two animals. Hence the average over the night was calculated over only six animals. NREM sleep was the predominant vigilance state during darkness (53.5% ± 7.7%). W was reduced to 25.6% ± 4.7%. REM sleep contributed 20.9% ± 3.7% to the recording time during darkness. In Fig. 2 the temporal distribution of the vigilance states over the 24 h period is plotted per hourly interval. The highest percentages of W were present in the first hours of the light period. During the course of the light period, time in W remained stable at around 75% until the last 2 h when it dropped to ca. 55%. During the first hour of the dark period animals were still awake for 52% of time. In the hour thereafter a steep decrease in the time awake could be observed. W remained around 15% until the last 2 h of darkness. In the last hour a steep increase in time awake occurred. Time asleep during light was mainly spent in NREM sleep. REM sleep contributed only 12.6% ± 8.6% (sd) to TST during the day. During the dark period time REM sleep made up 28.4% ± 6.1% of TST. No clear increase in REM sleep towards the end of the night could be observed. Maximum time in REM sleep was located in the interval 4 h before lights on; thereafter time in REM sleep decreased. Calculated as percentage of TST, REM sleep gradually increased from lights off until 4 h before lights on.

Power density. In Fig. 3 the time course of power density in the 1.5 Hz band during NREM sleep is plotted for one animal. During the light period

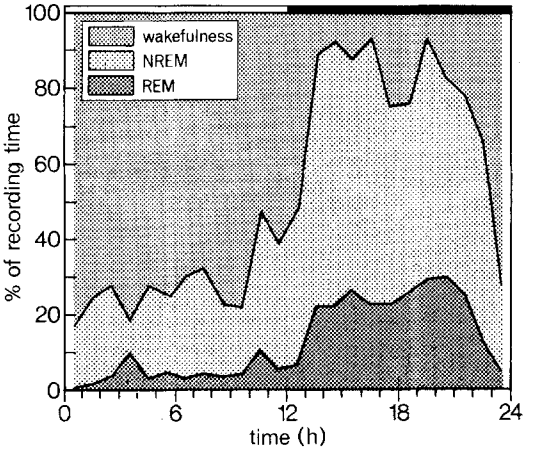

Fig. 2. Distribution of the three vigilance states over the 24-h period. Black bar indicates the dark period

a gradual increase in power density was seen. Maximum power density was reached 2 h after the beginning of darkness; thereafter power density steadily decreased. In Fig. 4 the average time course of power density (1.5 Hz) during NREM sleep of eight animals is plotted per hourly interval.

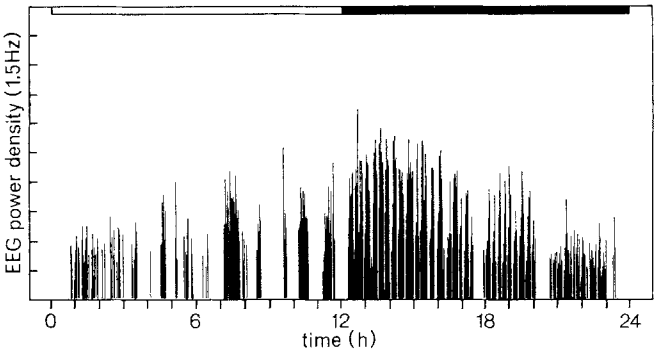

Fig. 3. EEG power density in the 1.5 Hz band-pass filter during NREM sleep in one animal

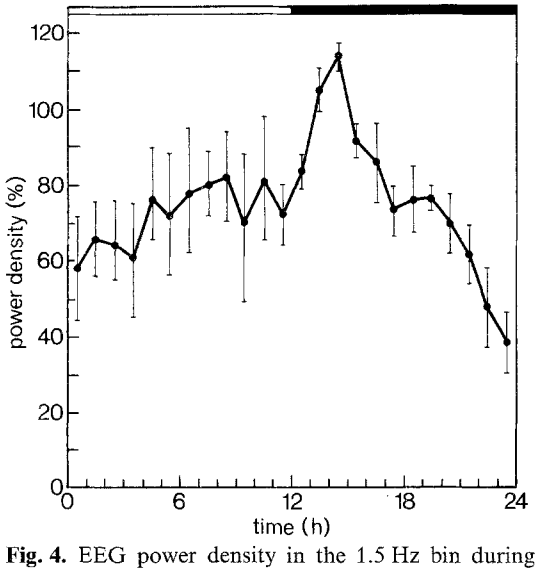

Fig. 4. EEG power density in the 1.5 Hz bin during NREM sleep. 100% = average power density during the first 4 h darkness; $n=8$, but not all animals contributed to the mean of all intervals. Vertical bars represent SEM

In each animal data were expressed relative to the power density in this frequency during NREM sleep in the first 4 h of darkness. Power density during the second part of the light period was somewhat higher than during the first part, but the increase was less pronounced than in the example shown in Fig. 3. During the first 2 h of the dark period a further increase was observed, followed by a decreasing course. The time course of power density in all frequencies was analyzed per 4-h interval. In each animal and each frequency bin power density was expressed relative to power density during the first 4 h of the dark period (Fig. 5). For statistical evaluation of the changes over the 24-h period, the relative power densities were subjected to a non-parametric ANOVA (Kruskal Wallis). Significant nonrandom variations over time were observed in the 0.75, 1.5, 2.5, 3.5, 8, 10, and 13.5 Hz bands. The direction of the trends was different for the lower and higher frequencies. Power densities in the lower frequencies increased during the light period and decreased during the dark period, whereas power densities in the higher freuqencies decreased during the light period and increased during the later part of the dark period.

Experiment 2: Photoperiods

Due to technical difficulties not all animals were recorded under all conditions, and not all recordings covered the entire 24-h period. Therefore, the number of animals contributing to the presented mean values varies among conditions and variables.

Vigilance states. The distribution of vigilance states over the 24-h period for the 3 light-dark regimes is depicted in Fig. 6. The data were calculated per 1-h intervals and double plotted to facilitate visual inspection. Under all three photoperiods the chip-

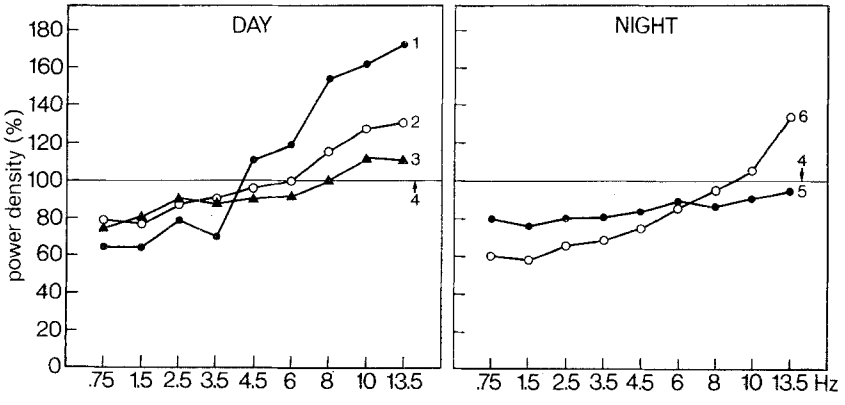

Fig. 5. EEG power density per frequency band during NREM sleep per 4-h interval. Data are expressed relative to the power density during the first 4 h of the dark period. Interval *1*: 0–4 h of the light period; interval *2*: 4–8 h of the light period, etc.

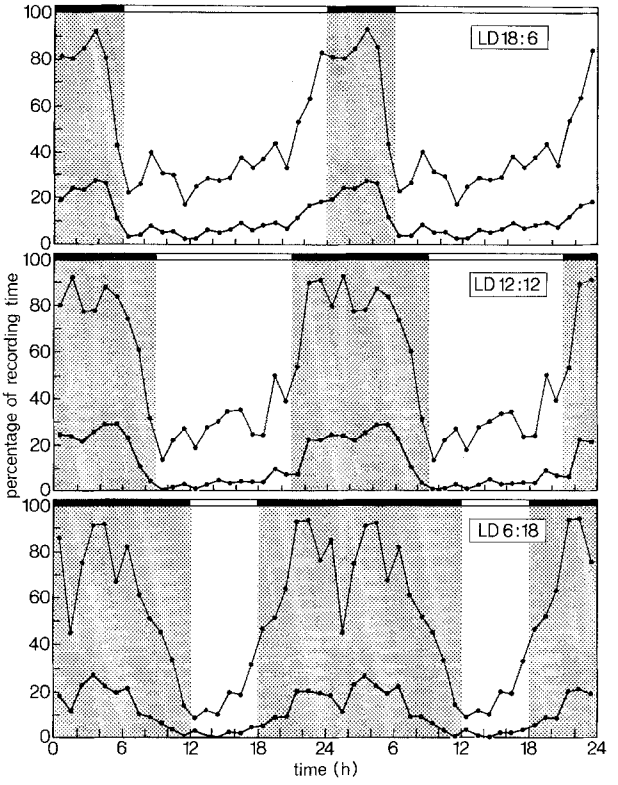

Fig. 6. Distribution of REM sleep (lower line), NREM + REM sleep (middle line), and wakefulness over the 24-h period under three different photoperiods. LD 18:6, $n=9$; LD 12:12, $n=9$; LD 6:18, $n=5$. Data are double plotted

munks' main rest phase coincided with the night. However, during the long days high percentages of sleep could be observed before lights off. During long nights, animals were awake for a considerable amount of time at the beginning and end of darkness.

The time course of REM sleep was also affected by the different photoperiods. During the short nights REM sleep increased towards the end of the night. During the long nights REM sleep gradually increased towards the middle of the night followed by a gradual decline. In Table 1 the effects of the different photoperiods on the distribution of TST and time in REM sleep over light and darkness are summarized. TST and REM sleep per 24 h are also presented. The different day lengths did not significantly affect TST per 24 h although TST was on average smallest under LD 18:6. Time in REM sleep per 24 h was not significantly affected either. The distribution of sleep over the light and dark periods was, however, significantly different across conditions. Highest percentages of sleep in the light were observed under LD 18:6. During darkness highest percentages of sleep were also observed in the LD 18:6 schedule. In the first 18-h interval, which in each condition started at lights on, time in NREM sleep was highest during the short days (see Table 1). So, although during the long photoperiods the animals slept more in the light, this increase in sleep time did not result in an equal amount of sleep during the first 18 h of the 24-h period. Hence, relative to the short photoperiods a NREM sleep deficit accumulated during the first 18 h of the long photoperiods.

Power density. The time course of EEG power density in the 1.5 Hz band during NREM sleep was analyzed for all three photoperiods (Fig. 7). In each animal and in each condition power densities were expressed as deviations from the mean power density per 24 h. Data were calculated per 2-h intervals. In all three conditions power density increased during the light and decreased during the dark period. This resulted in a long rising part of the power density curve during the LD 18:6

Table 1. Sleep stages during three photoperiods

	LD 18:6			LD 12:12			LD 6:18			H	$P<$
	x	sd	*n*	x	sd	*n*	x	sd	*n*		
TSTL	39.3	10.3	8	29.1	6.9	9	17.2	12.1	5	8.37	0.02
TSTD	77.6	9.5	9	74.7	4.4	7	65.7	4.7	4	6.61	0.05
TSTT	48.7	6.5	8	52.8	3.7	7	53.0	6.3	4	1.34	ns
REML	7.3	4.0	8	3.1	2.1	9	2.0	3.0	5	6.88	0.05
REMD	22.3	4.8	9	20.6	3.5	7	14.0	3.4	4	7.33	0.05
REMT	10.7	3.6	8	12.1	2.5	7	10.7	2.7	4	0.43	ns
NREM18	31.2	7.2	8	36.9	4.5	9	42.9	4.7	5	7.7	0.05

TSTL, total sleep time during the light period; *TSTD*, TST during the dark period; *TSTT*, TST averaged over day and night; *REML*, REM sleep during the light period, etc. *NREM18*, time in NREM during the first 18 h of the 24-h period. This interval begins in each condition at lights on. All values are expressed in percentage of recording time; *H*, Kruskal-Wallis, one-way, nonparametric ANOVA

schedule and a short rising part during the LD 6:18 schedule. The phase, relative to the moment of lights off, of highest power density was different for the three conditions. During the long photoperiod maximum power densities were located before the first hours of darkness, whereas under the short photoperiod maximum power densities were reached 3 h after lights off. Relative to the average power densities during darkness, power densities during the light period were highest under long days (ratios of power density day: power density night were 1.0 ± 0.13 (sd), 0.79 ± 0.17, and 0.75 ± 0.11 for LD 18:6; 12:12, and 6:18, respectively). During darkness the time course of power density did not parallel the time course of TST. Under all conditions, power density had already decreased before a decline in TST could be observed.

Experiment 3: Sleep deprivation

Vigilance states. During the baseline night the average percentages of time spent in the three vigilance states were $22.2\% \pm 4.6\%$ (sd) in W, $58.8\% \pm 5.9\%$ in NREM, and $18.1\% \pm 4.2\%$ in REM. During the night following 24 h of forced activity W was not significantly reduced ($20.9\% \pm 6.2\%$). Time in NREM sleep was not significantly changed either ($57.1\% \pm 7.2\%$). REM sleep was enhanced in six out of seven animals. The average percentage of time in REM sleep was $20.5\% \pm 4.1\%$ ($P = 0.075$ Wilcoxon matched pairs signed ranks test, baseline vs recovery).

Power densities. In Fig. 8 the power density (0.25–4.0 Hz) during baseline sleep and recovery sleep is plotted per 2-min period for one animal. In this example no clear progressive decay of power density over the baseline night was present. After sleep deprivation a decreasing trend could be observed in the second half of the night. The absolute value of EEG power density after sleep deprivation was higher than during baseline sleep.

The time course of power density (0.25–4.0 Hz) during sleep (NREM + REM) for the baseline night and recovery night averaged over all animals is depicted in Fig. 9. During the baseline night power densities in the delta frequencies decreased, although the decrease was not as impressive as in the first experiment (Fig. 4). After sleep deprivation delta power density was markedly enhanced, especially in the first 4 h of recovery sleep. The effect of sleep deprivation on power density was further analyzed by comparing power densities during recovery sleep to baseline values for 3-h

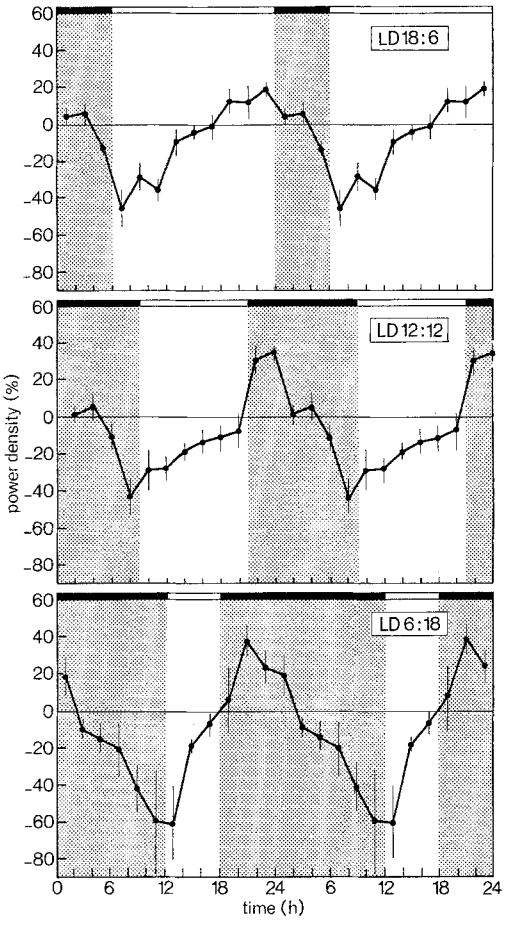

Fig. 7. Time course of EEG power density (1.5 Hz) during NREM sleep under three different photoperiods. Vertical bars represent SEM

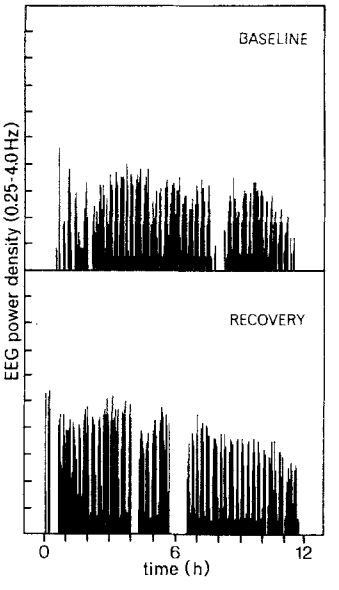

Fig. 8. EEG power density (0.25–4.0 Hz) during NREM + REM sleep in the dark period before and after sleep deprivation in one animal. Power densities are expressed in arbitrary units. Amplifications used in the two figures are identical

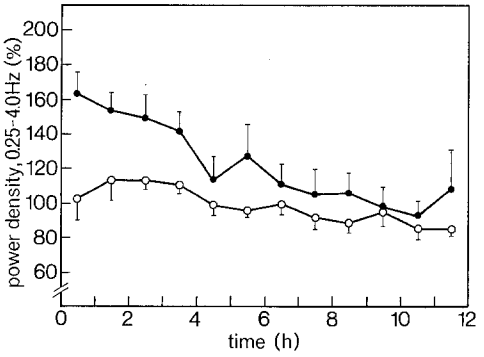

Fig. 9. EEG power density (0.25–4.0 Hz) during NREM + REM sleep before (open circles) and after (closed circles) sleep deprivation ($n=7$). Values are expressed relative to the average power density during the entire baseline night. Vertical bars represent SEM

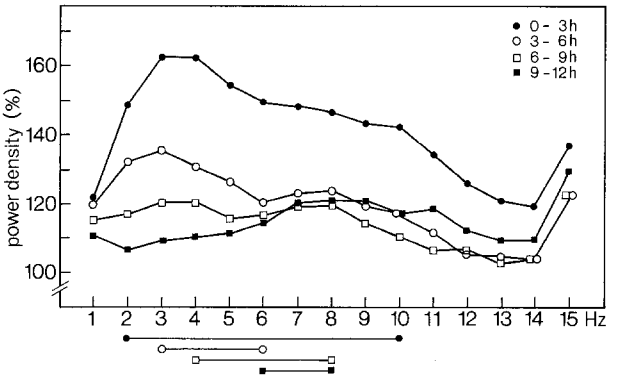

Fig. 10. Changes in power density during NREM and REM sleep after sleep deprivation. Values are expressed relative to power density in the corresponding interval of the baseline night. Horizontal bars below the x-axis: significant differences between recovery and baseline night ($P<0.05$, Wilcoxon matched pairs). Values are plotted at the upper limit of the frequency bins

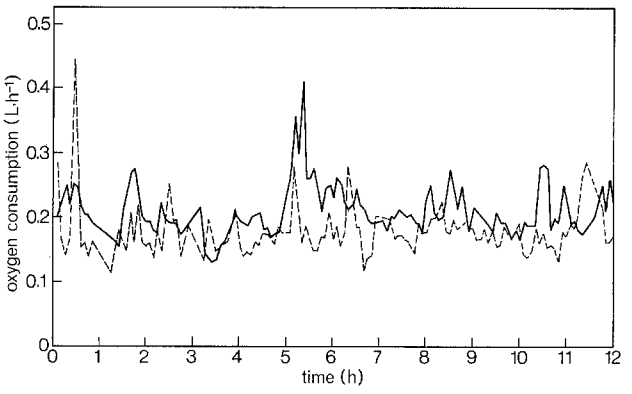

Fig. 11. Oxygen consumption of one animal plotted at 5-min intervals during the baseline night (broken line) and the first recovery night after sleep deprivation (solid line)

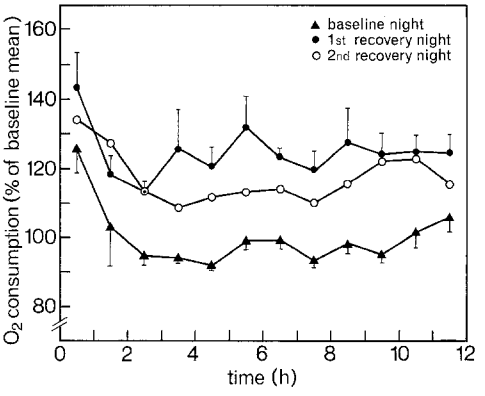

Fig. 12. Oxygen consumption plotted at hourly intervals during the baseline night ($n=6$), first recovery night ($n=6$), and second recovery night ($n=4$). All values are expressed relative to the mean oxygen consumption during the baseline night. Vertical bars represent SEM

intervals (Fig. 10). During the first 3 h of recovery sleep power densities were significantly elevated over a wide frequency range (from 1.25 to 10 Hz). The differences from baseline sleep became progressively smaller towards the end of the night. Nevertheless, significant enhancements of power densities in the theta frequencies (from 5.25–8 Hz), but not in the delta frequencies, were still present in the third and fourth 3-h intervals.

Experiment 4: Metabolic rate after sleep deprivation

In Fig. 11 the time course of oxygen consumption during the baseline and first recovery night is depicted for one animal. In both nights a considerable variation in O_2 consumption can be observed. It is likely that high values represent episodes of wakefulness whereas low values probably have been measured during sleep. During the recovery night the overall O_2 consumption was considerably higher than during the baseline night. Also, the lowest values in the recovery night exceeded those of the baseline night. Averaged over six animals oxygen consumption was 0.168 ± 0.006 (SE) $1\ O_2 \cdot h^{-1}$ during the baseline night and $0.207 \pm 0.005\ 1 \cdot h^{-1}$ during the first recovery night ($P<0.01$ paired t-test). In Fig. 12 oxygen consumption per hour expressed as percentage of the mean oxygen consumption of the whole baseline night is plotted at hourly intervals. During the entire recovery night values exceeded those of the baseline night. Even during the second recovery night oxygen consumption was still higher than during the baseline night although it was somewhat lower than during the first recovery night. In order to determine whether minimal O_2 consumption values were also affected for each night and each individual the 10 lowest 5-min values were determined. This analysis

showed that also minimum levels of O_2 consumption were increased after sleep deprivation [0.130 $l \cdot h^{-1} \pm 0.012$ (SE) and 0.158 ± 0.011 for baseline and first recovery night, respectively; $P < 0.01$; paired t-test].

Discussion

Under our experimental conditions TST in the Siberian chipmunk, expressed as a percentage of 24 h, is approximately 50%. Almost identical values are reported for the rat, but in the hamster TST is higher (Tobler and Jaggi 1987, Table 4; van Luijtelaar and Coenen 1984). TST as estimated by observation in *Eutamias dorsalis* and *Tamias striatus* as reported by Estep et al. (1978) was 62% and 68%, respectively. The predominance of W during the light period and of sleep during the dark period shows that the Siberian chipmunk is a day-active animal. Under LD 12:12 the ratio TSTD: TSTL is 2.83 whereas in the hamster the ratio TSTL:TSTD is 1.5. (Tobler and Jaggi 1987). In the rat ratios of 2.5 (Tobler and Jaggi 1987) and 1.88 (van Luijtelaar and Coenen 1983) have been reported. The ratio TSTD:TSTL in *Eutamias dorsalis* and *Tamias* was 1.20 and 1.04, respectively, as determined by observation (Estep et al. 1978). These differences in the temporal distribution of sleep are not necessarily species-specific. They may also be a consequence of housing conditions. In the present experiments the chipmunks had access to a running wheel. In most laboratory studies animals are kept singly in cages in which they have no access to a running wheel and do not have to work for their food. If the occurrence of sleep is indeed to a large extent determined by external conditions as is assumed in the two-process model, then it may not be very useful to compare TST of different species if they are not recorded under identical conditions.

There is a large difference in REM sleep, as percentage of TST in light and darkness. Similar differences have been reported for the rat and the hamster. In these nocturnal species the largest percentages are present in the light phase. As in the rat and hamster, in the chipmunk the percentage of REM sleep tends to increase towards the end of the major rest phase.

EEG spectral analysis revealed trends over the 24 h which were not apparent in the data based on visual scoring. Especially the power densities in the lower frequencies deserve attention. In the first experiment an increase in SWA in the course of the light period and a subsequent decline during the major rest phase were observed. This trend over the 24-h period again mirrors the situation in the rat (Rosenberg et al. 1976) and hamster. As in the rat (Trachsel et al. 1988) and hamster (Tobler and Jaggi 1987), power densities of the higher frequencies declined over the activity phase and tended to increase over the rest phase in the chipmunk.

Increments and decrements of the light period did not result in dramatic changes in TST per 24 h even though animals were exposed to the different photoperiods for several weeks. Similarly, Borbély and Neuhaus (1978) reported the absence of significant changes in TST in the rat when the light period was gradually extended. In the chipmunk, the distribution of sleep over light and dark was affected by the different photoperiods. The highest percentages of W were present at the beginning of the light period under all three photoperiods. In the later part of the long days TST was higher than in the later part of short days. TST during the dark and the time course of REM sleep are also dependent on the photoperiod. The enhancement of NREM sleep in the later part of the light period of the long days was not sufficient to obtain the amount of NREM sleep which accumulated during the first 18 h under short photoperiods. During the later part of the long days delta power density was, however, relatively high. This may represent a compensation in the intensity domain for the accumulated deficit in sleep time. The dissociation between the time course of TST during the dark period and power density indicates that these two sleep parameters are indeed regulated differentially. The mechanisms which are involved in the effects of photoperiod on the temporal distribution of sleep and wakefulness remain to be elucidated. A model of the circadian pacemaker proposed by Pittendrigh and Daan (1976) assumes that changes in photoperiod affect the phase relation between two oscillators (M and E) which constitute this pacemaker. These changes would result in changes in activity time. Changes in activity time with photoperiods have so far only been determined by measuring motor activity (cf. Daan and Aschoff 1975). Since bouts of motor activity may be interrupted by periods of sleep, such changes in activity time are not necessarily accompanied by changes in TST. The present data are, however, compatible with the view that in diurnal rodents, increments in the photoperiod lead to a lengthening of the interval in which activity prevails. This increase in activity time is compensated by high sleep intensity during the light as assessed by power density (Fig. 7) and high percentages of sleep during the night (Table 1).

In the third experiment the decline in delta power density (also called slow wave activity; SWA) during the rest phase was on average less prominent than in the first experiment although in five out of seven animals a clear decline was present. We do not have an adequate explanation for this difference between the two experiments. The time in NREM and REM sleep during the night was similar for the two experiments. Possibly the animals which did not exhibit a decline of SWA over the dark period slept more during the light period. Through more daytime sleep they may have reduced sleep pressure at the beginning of the dark period. This interpretation is supported by a 12-h sleep deprivation experiment (data not shown) in which the animals were sleep-deprived during the light period preceding the EEG recording. During the subsequent dark period we observed a steep decline in SWA. In Experiment 3 we found a similarly steep decline in SWA during the recovery night.

Twenty-four hours of sleep deprivation did not produce significant changes in TST. Time in NREM sleep was reduced somewhat whereas REM sleep was slightly enhanced. A larger REM rebound after 24 h of sleep deprivation is present in the rat (Borbély et al. 1984) whereas the hamster (Tobler and Jaggi 1987) showed a REM rebound similar to the chipmunk. At the beginning of the recovery night power densities were significantly enhanced compared with baseline values. Similar observations have been reported after 24 h sleep deprivation in the rat and the human (Borbély et al. 1984, 1981). In the hamster no significant enhancement of delta power density was reported after 24 h sleep deprivation, although 3 h sleep deprivation produced a massive enhancement of delta power density (Tobler and Jaggi 1987). The enhancement of power densities in higher frequencies cannot be explained by assuming that they are a monotonic function of prior W since in the course of the activity time the power densities in these frequencies decline in all three species. In the rat power densities in the higher frequencies were also enhanced after sleep deprivation (Borbély et al. 1984). These effects may be related to prolonged sleep deprivation in an unspecific way.

The results from experiment 4 do not support the hypothesis that an increase in SWA is accompanied by a further reduction in O_2 consumption. On the contrary, after sleep deprivation, O_2 consumption was higher than during baseline conditions. Although we did not record EEG and O_2 consumption simultaneously, it seems unlikely that in the O_2 consumption experiment no enhancement of SWA occurred since the experimental procedure was identical to the sleep deprivation experiment. It may be that the increase of O_2 consumption is a result of unspecific effects of the sleep deprivation procedure. In the rat forced activity does not result in a long-lasting elevation of corticosterone levels (Tobler et al. 1983), which indicates that the method of sleep deprivation may have been not particularly stressful. These considerations, however, are speculative, since we do not have either O_2 consumption data in rats or stress data in chipmunks. Comparing the effects of different sleep deprivation methods may solve this problem.

From the present experiments two major conclusions can be drawn. As in nocturnal rodents, in the diurnal chipmunk SWA increases during the activity phase and decreases during the rest phase. Since the activity and rest phases of diurnal and nocturnal animals differ 180° in phase relative to the light-dark cycle, these trends cannot be direct consequences of the absence or presence of light. They also cannot be attributed directly to the circadian pacemaker since in diurnal and nocturnal animals the phase relations between the LD cycle and the circadian pacemaker are similar, as shown by electrophysiological and behavioral studies (Sato and Kawamura 1984b; Hoban and Sulzman 1985). Since sleep and wakefulness are not uniformly distributed over the 24-h period, the hypothesis that SWA is a function of prior W and sleep can explain these trends. The finding that after 24 h sleep deprivation slow wave EEG activity is enhanced further supports this hypothesis.

The EEG data are qualitatively compatible with the two-process model of sleep regulation. The increase in SWA after sleep deprivation can be interpreted as a compensation for sleep loss by intensifying subsequent sleep. Since long and short photoperiods represent natural variations for these temperate zone animals, the responses of the EEG to photoperiod possibly reflect a natural adaptive compensation for changes in activity time. However, we suspect that changes of activity time with photoperiod may well be greater in the natural habitat than in our laboratory setup, since activity during the summer is probably extra enhanced by reproduction. Experiments in which variations in activity time and in sleep intensity are monitored simultaneously under a more naturalistic setting are needed to establish further the adaptive value of these regulatory mechanisms.

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References

Berger RJ (1984) Slow wave sleep, shallow torpor and hibernation: Homologous states of diminished metabolism and body temperature. Biol Psychol 19:305–326

Borbély AA (1982) A two process model of sleep regulation. Human Neurobiol 1:195–204

Borbély AA, Neuhaus HU (1978) Daily pattern of sleep, motor activity and feeding in the rat: effects of regular and gradually extended photoperiods. J Comp Physiol 124:1–14

Borbély AA, Neuhaus HU (1979) Sleep-deprivation: effect on sleep and EEG in the rat. J Comp Physiol 133:71–87

Borbély AA, Baumann F, Brandeis D, Strauch I, Lehmann D (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51:483–493

Borbély AA, Tobler I, Hanagasioglu M (1984) Effect of sleep deprivation on sleep and EEG power spectra in the rat. Behav Brain Res 14:171–182

Brebbia DR, Altschuler KZ (1965) Oxygen consumption rate and electroencephalographic stages of sleep. Science 150:1621–1623

Campbell S, Tobler I (1984) Animal sleep: a review of sleep duration across phylogeny. Neurosci Biobehav Rev 8:269–300

Daan S, Aschoff J (1975) Circadian rhythms of locomotor activity in captive birds and mammals: their variation with season and latitude. Oecologia 18:269–316

Daan S, Beersma DGM, Borbély AA (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Physiol 246:R161–R178

Dijk DJ, Beersma DGM, Daan S (1987a) EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythms 2:207–219

Dijk DJ, Beersma DGM, Daan S, Bloem GM, van den Hoofdakker RH (1987b) Quantitative analysis of the effects of slow wave sleep deprivation during the first 3 h of sleep on subsequent EEG power density. Eur Arch Psychiatr Neurol Sci 236:323–328

Estep D, Canney E, Cochran C, Hunter J (1978) Components of activity and sleep in two species of chipmunks: *Tamias striatus* and *Eutamias dorsalis*. Bull Psychon Soc 12:341–343

Frederickson CJ, Rechtschaffen A (1978) Effects of sleep deprivation on awakening thresholds and sensory evoked potentials in the rat. Sleep 1:69–82

Friedman L, Bergmann B, Rechtschaffen A (1979) Effects of sleep deprivation on sleepiness, sleep intensity, and subsequent sleep in the rat. Sleep 1:369–391

Grahnstedt S, Ursin R (1980) Awakening thresholds for electrical brain stimulation in five sleep-waking stages in the cat. Electroencephalogr Clin Neurophysiol 48:222–229

Hill RW (1972) Determination of oxygen consumption by use of the paramagnetic oxygen analyser. J Appl Physiol 33:261–263

Hoban TM, Sulzman FM (1985) Light effects on circadian timing system of a diurnal primate, the squirrel monkey. Am J Physiol 18:R274–R280

Inouye ST, Kawamura H (1979) Persistence of circadian rhythmicity in mammalian hypothalamic island containing the suprachiasmatic nucleus. Proc Natl Acad Sci USA 76:5962–5966

Mendelson WB, Vaughn WJ, Walsh MJ, Wyatt RJ (1980) A signal analysis approach to rat sleep scoring instrumentation. Waking and Sleeping 4:1–8

Pittendrigh CS, Daan S (1976) A functional analysis of circadian pacemakers in nocturnal rodents V. Pacemaker structure: a clock for all seasons. J Comp Physiol 106:333–355

Pohl H (1972) Die Aktivitätsperiodik von zwei tagaktiven Nagern, *Funambulus palmarum* und *Eutamias sibiricus* unter Dauerlichtbedingungen. J Comp Physiol 78:60–74

Pohl H (1976) Proportional effect of light on entrained circadian rhythms of birds and mammals. J Comp Physiol 112:103–108

Pohl H (1982) Characteristics and variability in entrainment of circadian rhythms to light in diurnal rodents. In: Aschoff J, Daan S, Groos GA (eds) Vertebrate circadian systems, structure and physiology. Springer, Berlin Heidelberg New York, pp 339–346

Rabiner LR, Gold B (1975) Theory and application of digital signal processing. Prentice Hall, Englewood Cliffs, NJ

Ramm P, Frost BJ (1986) Cerebral and local cerebral metabolism in the cat during slow wave sleep and REM sleep. Brain Res 365:112–124

Rosenberg RS, Bergmann BM, Rechtschaffen A (1976) Variations in slow wave activity during sleep in the rat. Physiol Behav 17:931–938

Rusak B, Zucker I (1979) Neural regulation of circadian rhythms. Physiol Rev 59:494–526

Sato T, Kawamura H (1984a) Effects of bilateral suprachiasmatic nucleus lesions on the circadian rhythms in a diurnal rodent, the Siberian chipmunk (*Eutamias sibiricus*). J Comp Physiol A 155:745–752

Sato T, Kawamura H (1984b) Circadian rhythms in multiple unit activity inside and outside the suprachiasmatic nuclei in the diurnal chipmunk (*Eutamias sibiricus*) Neurosci Res 1:45–52

Tobler I, Borbély AA (1986) Sleep EEG in the rat as a function of prior waking. Electroencephalogr Clin Neurophysiol 64:74–76

Tobler I, Jaggi K (1987) Sleep and EEG spectra in the Syrian hamster (*Mesocricetus auratus*) under baseline conditions and following sleep deprivation. J Comp Physiol A 161:449–459

Tobler I, Murison R, Ursin R, Ursin H, Borbély AA (1983) The effect of sleep deprivation and recovery sleep on plasma corticosterone in the rat. Neurosci Lett 35:297–300

Trachsel L, Tobler I, Borbély AA (1986) Sleep regulation in rats: effects of sleep deprivation, light and circadian phase. Am J Physiol 251:R1037–R1044

Trachsel L, Tobler I, Borbély AA (1988) Electroencephalogram analysis of non-rapid eye movement sleep in rats. Am J Physiol 255:R27–R37

Van Luijtelaar ELJM, Coenen AML (1984) An EEG averaging technique for automated sleep-wake stage identification in the rat. Physiol Behav 33:837–841

Zepelin H, Rechtschaffen A (1974) Mammalian sleep, longevity and energy metabolism. Brain Behav Evol 10:425–470