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Published in:

American journal of physiology-Regulatory integrative and comparative physiology

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

1998

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Strijkstra, A. M., & Daan, S. (1998). Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator. *American journal of physiology-Regulatory integrative and comparative physiology*, 275(4), R1110-R1117.

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Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator

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Strijkstra, Arjen M., and Serge Daan. Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator. *Am. J. Physiol.* 275 (Regulatory Integrative Comp. Physiol. 44): R1110–R1117, 1998.—Sleep regulation processes have been hypothesized to be involved in function and timing of arousal episodes in hibernating ground squirrels. We investigated the importance of sleep regulation during arousal episodes by sleep deprivation experiments. After sleep deprivation of 4, 12, and 24 h, starting 4 h after onset of euthermia, a duration-dependent enhancement of slow-wave activity (SWA) of the cortical electroencephalogram during non-rapid eye movement sleep was found, as expected for normal sleep regulation. When sleep deprivation was applied during the initial phase of the arousal episode, in which effects of prior torpor were present in undisturbed recordings, no subsequent recurrence of SWA was found. In addition, prior torpor induced a reduction in the spectral activity of the sigma frequency range (7–14 Hz), which was not observed after sleep deprivation. The effects of torpor and sleep deprivation on subsequent SWA appear qualitatively different. This indicates that effects of deep torpor on sleep are dissimilar to normal sleep regulation.

sleep regulation; electroencephalogram spectral analysis; ground squirrel; neuronal connectivity

THE HYPOTHESIS THAT euthermia during arousal episodes in hibernating mammals is required for sleep (7, 36) has received considerable attention in recent literature (4, 8, 17, 20, 31, 32). Experiments on several ground squirrel species have shown that animals mainly sleep during arousal episodes (*Spermophilus parryii*, Refs. 7 and 8; *S. lateralis*, Refs. 20 and 36; and *S. citellus*, Refs. 31 and 32). During the euthermic phase of arousal episodes, the relative power of slow waves [the slow-wave activity (SWA)] of the non-rapid eye movement (NREM) sleep electroencephalogram (EEG) decreases. This has been interpreted as a gradual reduction in sleep intensity during euthermia, which follows a hypothetical sleep debt accumulation during prior torpor (7, 36). Some evidence supports this interpretation: experiments have shown that a longer torpor duration resulted in higher initial SWA in the NREM sleep EEG in the early stages of the euthermic phase of arousal episodes in three ground squirrel species (8, 32, 36). Although these data are consistent with the idea that torpor generates a sleep debt, they do not prove it.

Larkin and Heller (20) found an initial peak in SWA in spontaneous arousal episodes at an ambient temperature of 11°C but not at 21°C. This suggests that the process generating high SWA is not involved in the generation of arousal episodes. We (31) investigated the effects of ambient temperature during torpor on subsequent sleep in induced arousal episodes after 4 days of

torpor; we found increased initial NREM sleep SWA during arousal episodes after torpor at an ambient temperature range of –5 to 10°C but found no increase at the ambient temperature of 15°C. Because the need to discharge SWA, and thus possibly sleep debt, does not increase during torpor at ambient temperatures of 15–21°C, and yet animals still arouse frequently, it is unlikely that sleep debt is a triggering factor in the mechanism of arousal episode occurrence.

However, during arousal episodes, these animals sleep for over 70% of the euthermic time. This sleep may still be of functional significance. It is conceivable that during the hibernation phase sleep regulation is organized in a different way compared with the continuously euthermic state. To investigate this, we performed sleep deprivations during arousal episodes to see if this manipulation would be followed by a rebound in sleep intensity (indicated by SWA). Sleep deprivation has been previously used in the Arctic ground squirrel (*S. parryii*). Barnes et al. (2) postulated that sleep deprivation during arousal episodes would lead to a subsequent need to reduce the accumulated sleep debt before reentry into torpor, thus lengthening the arousal episode. Indeed, they observed lengthening of arousal episodes after sleep deprivation. However, the interpretation of the results is ambiguous, since the effects of sleep deprivation were not evaluated with EEG recordings and the lengthening of the arousal episode far exceeded expected lengthening requirements for sleep satiation. We addressed the question of whether the effect of sleep deprivation on subsequent sleep is similar to the effect of prior torpor. A direct comparison of the two effects could settle the issue of whether both effects on SWA are of the same nature.

In the present study, we sleep deprived European ground squirrels during the euthermic phase of arousal episodes, varying both timing and duration of the sleep deprivation. We recorded EEGs during both uninterrupted arousal episodes and recovery from sleep deprivation. The results suggest that the initially high SWA, which is due to effects of prior deep torpor, is not attributable to normal sleep regulation. Later, in the arousal episode, sleep regulation appears to act in a normal way.

METHODS

Animals. Recordings from nine female European ground squirrels (*S. citellus*) were made over the 1993–1994 hibernation season. The animals were caught in the wild in the summer preceding hibernation from a population near Vienna, Austria. Before hibernation, the animals were individually housed in 60 × 40 × 40 cm (high) steel cages with sawdust bedding, in a temperature-controlled room (20 ±

1°C, 12:12 h light-dark cycle). Hay was provided as nesting material. The animals were fed ad libitum with commercial rabbit food pellets (Teurlings). In late September, silver EEG electrodes and stainless steel electromyogram (EMG) electrodes (MS303, Plastics One, Roanoke, VA) were implanted under halothane anesthesia (2.5%, in air). EEG electrodes were placed on the dura above the parietal cortex and the cerebellum. Two screws on the frontal cortex served as ground. Eight animals were simultaneously equipped with a thermistor on the dura above the frontal cortex. The thermistor was sealed in a glass capillary (outer diameter of 1.0 mm) and calibrated before implantation. All leads were fixed to a connector and mounted on the skull with dental cement.

Recordings. In early October, the environment was changed to an ambient temperature of $6 \pm 1^\circ\text{C}$ and continuous dim light (<1 lux). Seven animals had already shown torpor before the ambient temperature change. All animals were hibernating by the end of October. From early November on, the animals were checked daily for occurrences of spontaneous arousal episodes by visual inspection or touch: animals were considered to have had an arousal episode during the previous day if the animal was warm and/or the position in the nest had changed since the previous check. When the animal was found torpid the next day, this was considered the first day in torpor. EEGs were recorded from early December to early March, when spontaneous torpor bout duration is relatively stable. Animals were induced to arouse by gentle handling at room temperature after 4 days of torpor, to standardize prior torpor duration. This particular torpor duration was used because the putative need for SWA was expected not to be at the maximum level that is eventually reached after longer torpor (32). EEGs were recorded by connecting the animal's implanted electrodes via a slip ring swivel (Air Precision, Le Plessis Robinson, France) to an amplifier system (3T, Enschede, The Netherlands; EEG: 200 mV/V, 0.2–80 Hz, EMG: 500 mV/V, 20–600 Hz). The amplified EEG and EMG signals were digitized and sampled by the data acquisition and analysis program POLY (Inspector Research Systems, Amsterdam, The Netherlands) at 100 Hz, filtered by a software low-pass filter (–3 dB at 17 Hz, –35 dB per octave) and analyzed online by fast Fourier transformation per 10-s interval. The signal was stored at 20 Hz for later visual scoring of wakefulness (wake) and NREM sleep and rapid eye movement (REM) sleep. Average spectral EEG power was calculated per 2-h interval for 0.2–15 Hz in 1-Hz bins and for the slow-wave frequency range (1.2–4 Hz). Power was expressed relative to EEG power recorded over a standard time and situation available for all animals: the average power of the EEG frequency range during the first 6 h of euthermy of the undisturbed baseline recording. This was done to correct for differences between individuals. This calculation yielded EEG activity values per 1-Hz frequency bin and for the slow-wave range (SWA).

Sleep deprivation. Sleep deprivation was applied to obtain information on the similarity between the effects of sleep deprivation (prior wakefulness) in euthermy on subsequent sleep EEG and the effects of prior torpor on the subsequent sleep EEG. First, to assess the time course of the effects of sleep deprivation during euthermy wakefulness, the animals were allowed to sleep for the first 4 h in the euthermy phase of the arousal episode. Previous studies (31, 32) showed that the duration-dependent effects of prior torpor on SWA were confined to this phase in similar circumstances. Subsequently, the animals were sleep deprived for 4 h (*treatment 1a*, $n = 8$), 12 h (*treatment 1b*, $n = 9$), and 24 h (*treatment 1c*, $n = 7$) by forced locomotion in a slowly rotating drum (50 cm diameter, 0.25 rpm). During sleep deprivation by forced

locomotion, ambient conditions were similar to those during EEG/EMG recordings, i.e., continuous dim light, 6°C, and water ad libitum. Because animals did not appear to eat during the phase of hibernation in which we recorded EEG/EMG, no food was supplied in the rotating drum. After sleep deprivation, the animals were returned to their home cages for the recovery EEG/EMG recording. The recording lasted, in all but two cases, until the animal had reentered torpor.

To assess the significance of the torpor-induced high initial SWA in the first euthermy 4 h of arousal episodes (0–4 h), we performed sleep deprivations during this phase of the arousal episode by forced locomotion (*treatment 2*, $n = 9$). The recovery data were compared with recovery data after sleep deprivation of the same duration (4 h) but starting after 4 h of euthermy (4–8 h, *treatment 1a*, $n = 9$). In a subset of the animals, we repeated these sleep deprivation experiments with the same timing but with the handling method. This procedure allowed us to record EEG/EMG during sleep deprivation. Six animals were sleep deprived during the first 4 h of euthermy (*treatment 3a*) and five animals during 4 h but starting after 4 h in euthermy (*treatment 3b*). In the handling procedure, a computer screen displaying the online EEG and EMG traces was placed adjacent to the animal's cage to check the vigilance state of the animal. The animal was disturbed by tactile stimuli when it started to show an NREM sleep EEG pattern. Light emission of the monitor was maximally reduced and was below 1 lx at the animal's position. Light emission directed to the animal was blocked by cloth.

Sleep deprivation was performed starting at different phases of the arousal episode. This required an ad hoc decision on the timing of the onset of the euthermy phase of the arousal episode during the measurement. The end of the initial EMG surge was used as an indicator: in previous studies, we have used this as a post hoc marker of the beginning of the euthermy phase (31, 32). The time of reentry into torpor was defined as the time of the last substantial wake bout before the EEG power decreased by a temperature effect. These measures were shown to be closely related to the occurrence of euthermy cortical temperatures (31, 32).

For statistical evaluation, we used Kruskal-Wallis nonparametric ANOVA (KWNPA) for variation between multiple groups and Mann-Whitney's rank sum test (MWU), Wilcoxon's signed rank test (WSR), and Spearman's rank correlation (SRC) for correlations.

RESULTS

Torpor. Individual spontaneous torpor bout durations ranged from 7.3 to 11.6 days and averaged 9.4 ± 1.3 days (SD; $n = 9$). For the EEG/EMG recordings, animals were induced to arouse after 4 days and thus well before their spontaneous arousal episode was expected. Individual (rectal) body temperatures (T_b) of the animals in torpor, measured when the animals were picked up for initiating the arousal episode, ranged from 7.4 to 8.7°C and averaged $7.9 \pm 0.4^\circ\text{C}$ (SD; $n = 9$). Torpid cortical temperature (T_{crt}), measured simultaneously, ranged from 6.7 to 7.9°C and averaged $7.4 \pm 0.4^\circ\text{C}$ (SD; $n = 8$).

Arousal episode. Because of technical difficulties, we did not obtain T_{crt} for all EEG/EMG recordings. From the available T_{crt} recordings, we derived estimates of the accuracy of relying on EMG measures for determining the euthermy phase duration (EPD), which was the method used for all recordings. We took the lowest

2-min average T_{crit} between the first substantial wake bout following the initial warming-up EMG surge and the last substantial wake bout before the EEG power decreased by temperature effects. Between the first and last substantial wake bout, there were no fluctuations in T_{crit} that could indicate reentry into torpor. A comparison of T_{crit} values at the end of the EMG surge [$29.3 \pm 0.6^\circ\text{C}$ (SE) $n = 8$] showed that the animals ended the initial EMG surge 24.9 ± 1.9 min (SE; $n = 8$) before the euthermic level of T_{crit} [$32.2 \pm 0.3^\circ\text{C}$ (SE), $n = 8$] was reached. In previous experiments (31, 32), the time lag averaged 10–12 min. The difference between the previous experiments and the present experiments could be due to the ad hoc nature of the assessment of the end of EMG activity in the present data. The longer time lag might have caused the first 2-h interval EEG power to be affected to a larger extent compared with the previous experiments. To investigate this, we compared average slow-wave power in the first 2-h interval, both including and excluding values before the euthermic T_{crit} level was reached. Including the slow-wave power data before the euthermic T_{crit} level slightly reduced the average SWA by $5.8 \pm 8.8\%$ (SD; $n = 8$). As in the previous experiments, this effect did not reach significance (WSR: $P = 0.14$).

The end of the euthermic phase was defined as the timing of the last substantial activity bout, occurring before the sleep bout wherein EEG power gradually declines, as the animal reenters torpor. The T_{crit} at the estimated timing of the end of euthermia [$32.7 \pm 0.2^\circ\text{C}$ (SE), $n = 7$] was close to the lowest euthermic T_{crit} level.

EPD: effect of sleep deprivation. EPD of both the undisturbed baseline recordings and the recordings including sleep deprivation are shown in Table 1. Experimental sleep deprivation obviously lengthened

Table 1. EPD of undisturbed baseline arousal episodes and arousal episodes when sleep deprivation occurred under various methods, durations, and timings

Sleep Deprivation				n	EPD, h	Range of EPD, h	Corrected EPD, h	P
Duration	Method	Timing						
Baseline				9	8.7 ± 1.5	6.7–11.1	8.7	
4 h	HG	0–4 h		6	11.5 ± 2.0	8.9–14.2	7.5	0.38
4 h	HG	4–8 h		5	11.5 ± 1.6	8.9–13.2	7.5	0.42
4 h	FL	0–4 h		9	11.8 ± 2.7	9.1–16.9	7.8	0.33
4 h	FL	4–8 h		8	12.9 ± 2.7	10.4–17.8	8.9	0.81
12 h	FL	4–16 h		9	21.7 ± 2.2	18.8–26.3	9.7	0.33
24 h	FL	4–28 h		7	37.4 ± 7.3	32.4 to >49.0	13.4	0.044

Values are means \pm SD; $n =$ no. of animals. For all experiments prior torpor duration was 4 days. Euthermic phase duration (EPD) results are given with the assumption that animals were euthermic during all sleep deprivation experiments. Sleep deprivation was performed by handling (HG) the animal when it started to show a non-rapid eye movement (NREM)-like electroencephalogram (EEG) or by forced locomotion (FL) in a slowly rotating drum. Corrected EPD excludes the duration of the imposed sleep deprivation. In the 24 h sleep deprivation group, two recordings were stopped before the animals returned to euthermia for practical reasons. Both animals were euthermic for >49 h. These data were included in the nonparametric test but are not represented in the average values. P values indicate significance of differences between the corrected EPD and the baseline EPD tested by Mann-Whitney's rank sum test.

Table 2. Vigilance states during the euthermic phase of arousal episodes following sleep deprivation under various methods, durations and timings

Sleep Deprivation				Vigilance State, %		
Duration	Method	Timing	n	Wake	NREM	REM
Baseline			9	19.6 ± 3.5	69.1 ± 3.3	11.4 ± 2.2
4 h	HG	0–4 h	6	23.3 ± 8.0	$63.1 \pm 4.7^*$	13.6 ± 4.0
4 h	HG	4–8 h	5	29.9 ± 8.4	$57.8 \pm 1.9^\dagger$	12.4 ± 7.4
4 h	FL	0–4 h	9	20.5 ± 5.2	66.8 ± 3.9	12.6 ± 3.5
4 h	FL	4–8 h	8	21.7 ± 5.2	65.7 ± 4.3	12.6 ± 1.8
12 h	FL	4–16 h	9	19.0 ± 4.6	$64.3 \pm 4.8^*$	$16.7 \pm 1.8^\dagger$
24 h	FL	4–28 h	7	20.1 ± 7.7	63.4 ± 6.6	$16.5 \pm 2.0^*$
				0.35	0.0033	0.0009

Values are means \pm SD; $n =$ no. of animals. Arousal episodes were induced after 4 days of torpor. Percentages of wakefulness (wake), NREM sleep, and REM sleep are given for undisturbed baseline recordings and recovery recordings after sleep deprivations. Sleep deprivation of 4 h was carried out commencing from the start of euthermia (0–4 h) or 4 h later in the euthermic phase either by handling (HG) or forced locomotion in a slowly rotating drum (FL). Sleep deprivation of 12 and 24 h were done with FL starting 4 h after start of euthermia. For 24 h sleep deprivation group, two recovery recordings were stopped before the animals reentered torpor for practical reasons. P values represent results of comparing all categories with Kruskal-Wallis nonparametric ANOVA. Significant differences from the baseline record tested by Mann-Whitney's rank sum test are as follows: * $P < 0.05$; $\dagger P < 0.01$.

the total EPD. This is in part a trivial result, since the sleep deprivation manipulation by itself artificially lengthens euthermia. The reaction of the animals to the manipulation may be better represented by the length of the undisturbed fraction of the total EPD, i.e., EPD of which the sleep deprivation duration is subtracted. These values are given as "corrected" EPD values in Table 1. The P values in Table 1 represent test results of comparisons made between the corrected EPD values of the manipulated groups and the undisturbed baseline EPD values. Thus sleep deprivations of 4 or 12 h on average lengthened the EPD by approximately as much time as the duration of the sleep deprivation itself; after subtraction of the 4- or 12-h sleep deprivation duration, there was no significant difference from baseline EPD. The 24-h sleep deprivation on average lengthened the corrected EPD; in two cases, the animals even stayed euthermic for more than 20 h following the 24-h sleep deprivation. These recordings had to be ended before the animals reentered torpor for practical reasons. The animals were found torpid 1–2 days later. In the nonparametric test of a difference of corrected EPD between the various sleep deprivations compared with the baseline EPD, we assigned the (conservative) value of 20 h as the corrected EPD value for these two animals. In the comparisons between baseline EPD and corrected EPD of the various sleep deprivation categories, a difference was found between the corrected 24-h sleep deprivation EPD and the baseline EPD (Table 1, MWU: $P = 0.044$).

Vigilance states during euthermia. The percentage of euthermic time spent awake or in NREM and REM sleep are given for baseline and recovery recordings for the different treatments in Table 2. The percentage of total sleep time did not significantly vary between

treatments (KWNPA: $P = 0.35$). Also, in direct comparison to baseline, no significant differences in total sleep time were encountered in any recovery recording category. In direct comparison to baseline, NREM sleep percentage was reduced after sleep deprivation that was brought on by handling. This reduction was not significant in comparison to the other sleep deprivation treatments. In comparison to baseline, REM sleep percentage was increased after 12 and 24 h of sleep deprivation.

Spectral analysis. To investigate EEG changes, we used spectra averaged per 2-h interval over a 0.2- to 15-Hz range per 1-Hz bin for the NREM sleep EEG. We expressed EEG power for the specific frequency bin relative to the NREM sleep EEG power in that bin, averaged over the first 6 h of the baseline recording for each individual. The spectra for the first three 2-h intervals for all sleep deprivation recovery recordings are shown in Fig. 1. First, we investigated where changes in spectral power occurred as a function of time within the different treatment categories. For the baseline condition, significant variation was found for 1.2–5 Hz (KWNPA: $P < 0.05$); that is, a decrease was observed; for higher frequencies [7.2–14 Hz (KWNPA: $P < 0.05$)], an increase was observed. For the recovery recordings after 4 h of sleep deprivation (*treatments 1a, 2, 3a, and 3b*), no significant variation in time was observed in any frequency bin. Also after 12-h sleep deprivation, no significant variation over time was found in the recovery recording in any frequency bin, although the spectral power in the lower frequencies (~1.2–4 Hz) was high compared with baseline (see Fig. 1), and a subsequent reduction thus might have been expected. This lack of effect may be caused by the reduction of animals that were euthermic in the later 2-h intervals (for the fourth 2-h interval, $n = 3$ of 9 individuals; for the fifth 2-h interval, $n = 2$). Significant variation over time was found in the recovery recordings after 24 h of sleep deprivation for the 2.2- to 5-Hz frequency range (KWNPA: $P < 0.05$), in which a reduction in activity was observed. The proportion of

animals that stayed euthermic for a longer time and thus contributed to the correlation was higher than in the recovery recordings after 12 h of sleep deprivation (for the fourth to seventh 2-h interval, $n = 3$ out of 7 individuals; 2 animals stayed euthermic for over 20 h after sleep deprivation, see *EPD: effects of sleep deprivation*).

We compared the effects of torpor on the EEG spectra, expressed in the uninterrupted baseline condition, with the effects of induced wakefulness, expressed in the recovery recordings of sleep deprivation treatments. Data of baseline and recovery recordings combined showed significant variation in spectral activity over the entire spectrum (0.2–15 Hz, KWNPA: $P < 0.01$, 53 cases) in the first 2-h interval (see Fig. 1). Effects were restricted to a low-frequency range in the second 2-h interval (0.2–4 Hz, KWNPA: $P < 0.01$, 52 cases) and the third 2-h interval (0.2–3 Hz, KWNPA, $P < 0.05$, 45 cases). In the fourth 2-h interval, no significant variation was observed (27 cases, not shown). Most of the variation in the high frequencies in the first 2-h interval seemed to be related to the baseline condition (see Fig. 1). Exclusion of the baseline recordings restricted significant variation to a low-frequency range (0.2–7 Hz, KWNPA, $P < 0.05$, 44 cases). The data indicate that spectral changes in the frequency range of 7.2–14 Hz are restricted to the early stage of the euthermic phase, as an effect of prior torpor. The 7.2- to 14-Hz frequency range was not modified by euthermic sleep deprivation. Effects of euthermic sleep deprivation on the spectrum were restricted to the 0.2- to 7-Hz frequency range.

Sleep regulation during arousal episodes. In *treatments 1a, 1b, and 1c*, sleep deprivations of 4, 12, and 24 h were performed by forced locomotion. Sleep deprivation was started after 4 h of euthermia, to avoid mixing the effects of euthermic sleep deprivation with the effects of torpor. Figure 2 compares SWA in the first four 2-h intervals of the baseline recordings with SWA in the first two 2-h intervals of recovery recordings following euthermic sleep deprivations of 4, 12, and 24 h. SWA

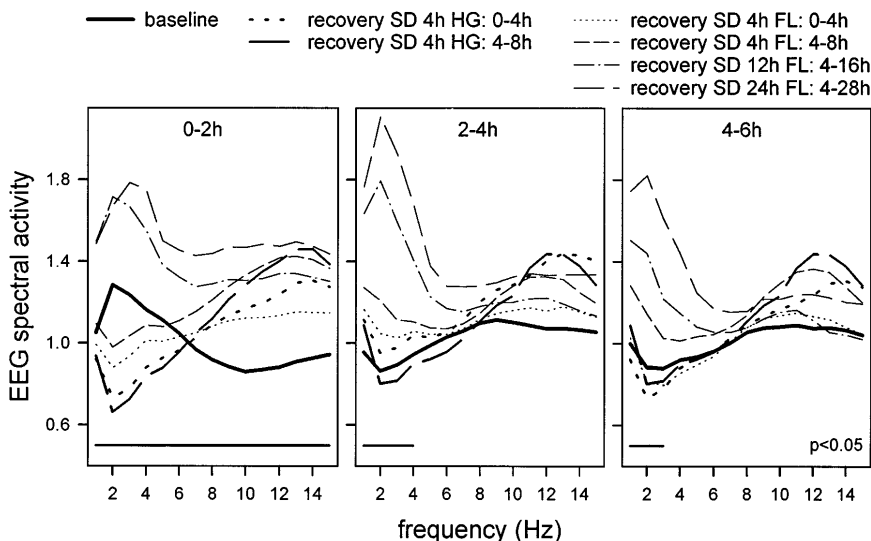


Fig. 1. Non-rapid eye movement (NREM) sleep electroencephalogram (EEG) spectral activity during the first three euthermic 2-h intervals following 4 days of torpor (baseline) or various euthermic sleep deprivation (SD) treatments (recovery). Spectral activity was calculated per 1-Hz frequency bin (0.2–1 Hz, 1.2–2 Hz, etc.) and expressed relative to the power of the 1-Hz frequency bin over the first 6 euthermic hours of the baseline recording. Sleep deprivation was performed by handling (HG) or forced locomotion (FL). Sleep deprivation duration was 4, 12, or 24 h. Sleep deprivation was started either immediately after onset of euthermia (0–4 h) or after 4 h of euthermia (4–8, 4–16, 4–28 h). Horizontal lines (*bottom*) indicate significant variation between all treatment categories (Kruskal-Wallis nonparametric ANOVA: $P < 0.05$).

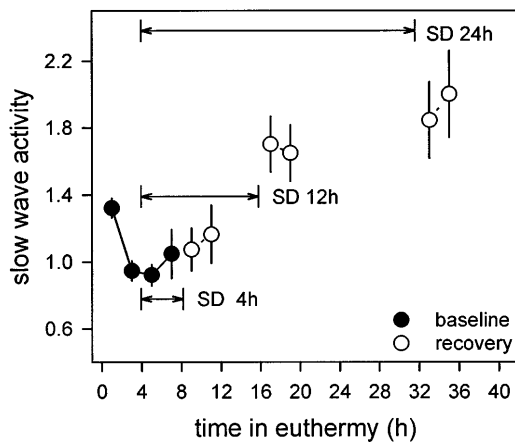


Fig. 2. NREM sleep EEG slow-wave activity (SWA) of baseline arousal episodes and following euthermic sleep deprivation (SD). Sleep deprivation was done by forced locomotion. ●, First four 2-h intervals of the undisturbed baseline SWA. ○, SWA after sleep deprivation of 4, 12, or 24 h. Timing of sleep deprivation is indicated by arrows.

showed a positive association with the duration of euthermic sleep deprivation (SRC: $r^2 = 0.62$, 24 cases, $P < 0.005$). An exponentially saturating curve fitted to these data yielded the descriptive model $SWA = 0.75 + 1.63e^{-0.27/t(n)}$ ($F_{2,23} = 6.55$; $P = 0.0061$), where t = sleep deprivation duration in hours.

Sleep regulation and torpor. To investigate the similarities of the SWA increase caused by prior torpor and by euthermic wakefulness, we compared the effects of sleep deprivations of 4-h duration (treatments 1a, 2, 3a, and 3b). Sleep deprivation was performed by forced locomotion or handling. For one individual, T_{crit} and concurrent SWA are shown throughout the baseline recording (Fig. 3A), during the recordings in which sleep deprivation was performed by handling (Fig. 3, B and C), and before and after the 4-h sleep deprivation by forced locomotion (Fig. 3, D and E). In both the baseline recording and the recordings in which the first 4 h of euthermy was not influenced by sleep deprivation (Fig. 3, C and E), initial SWA was high. SWA during the recordings after 4 h of sleep deprivation appeared similarly independent of the timing of sleep deprivation, for cases in which sleep deprivation was performed by both handling (Fig. 3, B and C) and forced locomotion (Fig. 3, D and E).

Figure 4 shows the average SWA of all the recordings following 4 h of sleep deprivation for the baseline recordings and the recovery recordings from the differentially timed 4 h of sleep deprivation by forced locomotion and handling. During sleep deprivation induced by handling, NREM sleep percentage was reduced compared with baseline, from $73.4 \pm 3.1\%$ (SD, $n = 6$) to $13.1 \pm 5.4\%$ (SD, $n = 6$; MWU: $P = 0.0051$), and REM sleep percentage was almost entirely suppressed, from $6.8 \pm 1.2\%$ (SD, $n = 6$) to $0.1 \pm 0.3\%$ (SD, $n = 6$; MWU: $P = 0.0051$). SWA during the remaining NREM sleep was reduced to 0.48 ± 0.11 (SE; MWU: $P < 0.005$) and was similar for sleep deprivation results during 0–4 and 4–8 h of euthermy. During the recovery recordings after the sleep deprivation in the first 4 h, and thus

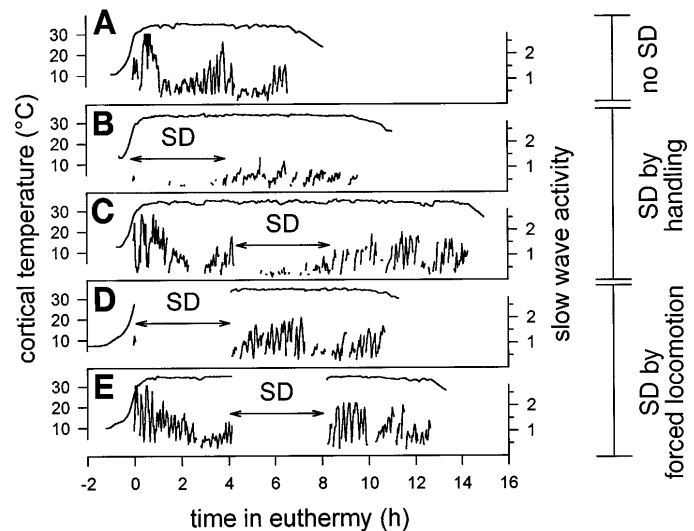


Fig. 3. Cortical temperature (T_{crit}) and concurrent NREM sleep EEG SWA of *S. citellus* (no. 52) during undisturbed baseline recording (A), during recordings where 4 h of sleep deprivation (SD) was performed by handling (B and C), and before and after 4 h of sleep deprivation by forced locomotion (D and E). After induction of arousal episode, the animal warmed up to euthermic T_{crit} levels. Onset of euthermy (time in euthermy = 0) was defined as the end of shivering EMG; end of euthermy was defined as the time of the last activity bout before EEG power declined by a temperature effect. SWA is shown for the euthermic phase. Undisturbed SWA during the first 4 h of euthermy was high (A, C, and E). SWA levels in recovery recordings after 4 h of sleep deprivation appeared similar when sleep deprivation was applied in hours 0–4 of euthermy or hours 4–8, for both methods of sleep deprivation. Expected recurrence of the high initial SWA after sleep deprivation during 0–4 h did not occur.

compromising the expression of initial high SWA in NREM sleep, a recurrence of SWA of similar magnitude (or larger) was expected. However, compared with the recovery following sleep deprivation during hours 4–8 of euthermy, there was no significant difference in

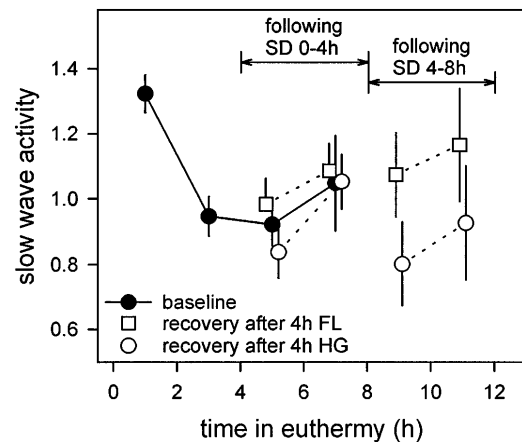


Fig. 4. NREM sleep EEG SWA of the first two 2-h intervals following differently timed 4-h sleep deprivation (SD). Sleep deprivation was done by handling (HG) or forced locomotion (FL) during 0–4 or 4–8 h of time in euthermy. ●, Undisturbed baseline SWA. ○, SWA following sleep deprivation by handling. □, SWA following sleep deprivation by forced locomotion. No significant differences were observed between 1) baseline SWA and SWA following sleep deprivation during 0–4 h of time in euthermy and 2) SWA following sleep deprivation during 0–4 h and SWA following sleep deprivation during 4–8 h of time in euthermy.

SWA. This was true for both the forced locomotion sleep deprivation (MWU: $P = 0.51$) and the handling sleep deprivation procedures (MWU: $P = 0.70$). Also, in direct comparison to SWA in *hours 4–8* of the undisturbed baseline recordings, no significant differences were encountered compared with recovery after 4 h of sleep deprivation by either method (MWU: $P > 0.17$). These data indicate that blocking the expression of the initial posttorpor increase in SWA by sleep deprivation did not result in a subsequent compensatory rise in SWA in *hours 4–8*.

DISCUSSION

The present data show first that sleep deprivation starting 4 h after the onset of euthermy in arousal episodes leads to a sleep deprivation duration-dependent increase in SWA in hibernating ground squirrels. Thus sleep regulation appears to function in a normal way during arousal episodes. It is therefore reasonable to make direct comparisons between effects of euthermy sleep deprivation and the effects of torpor, to test the hypothesis that arousal episodes serve a sleep regulatory function. Second, in direct comparison to effects of prior torpor vs. euthermy sleep deprivation on subsequent NREM sleep EEG variables, torpor not only enhanced SWA but also reduced activity of higher frequencies. Third, sleep deprivation during the initial phase of the arousal episode did not postpone the expression of initial high SWA that normally occurs in that phase of the arousal episode. This indicates that, when SWA is enhanced by the effects of prior torpor, sleep is not required to reduce SWA. The results provide evidence that the effects of prior torpor on the EEG are qualitatively different from effects of euthermy sleep deprivation.

The baseline data were in many respects similar to the baseline data of prior experiments (31, 32). T_b and T_{crit} during torpor, the EPD, the amount of time spent sleeping, the initial SWA increase after 4 days of torpor, and the initial decrease in higher frequencies were similar, also in comparison to another ground squirrel species (36). The effects of torpor on sleep during arousal episodes thus appear robust.

EPD was affected by sleep deprivation in all cases. For 24-h sleep deprivation experiments, the EPD was lengthened more than could be expected, i.e., more than the duration of an uninterrupted arousal episode enlarged by 24 h. A 12-h sleep deprivation did not enlarge the EPD more than expected. An interesting result was found when 4 h of sleep deprivation was applied. The animals returned to torpor after a certain amount (~8 h, approximately the length of the baseline recordings) of undisturbed time in euthermy, which was independent of the timing of the sleep deprivation disturbance. If the sleep deprivation was immediately started at onset of euthermy, the animals would return to torpor after ~8 h of continuous undisturbed euthermy following sleep deprivation. If the sleep deprivation was started after 4 h of euthermy, animals would return to torpor after another ~4 h of undisturbed euthermy following sleep deprivation. Hence, it was not the

length of the euthermy interval between sleep deprivation and reentry into torpor that was specifically defended but rather the overall undisturbed resting time during an arousal episode. Possibly, processes involved in the function of arousal episodes have to be performed (or are facilitated) during undisturbed resting time in euthermy. Alternatively, the effect may be related to other unintended phase-specific effects of the sleep deprivation procedure, which not only suppresses sleep but may also induce stress or may enhance exposure of the eyes to the available dim (<1 lx) light.

Effects on the NREM sleep EEG induced by prior torpor appeared to be qualitatively different from effects induced by euthermy sleep deprivation. Differences in spectral activity occurred in the NREM sleep EEG: both torpor and sleep deprivation resulted in an increase of SWA (1–4 Hz), but torpor also affected sigma frequencies (7–14 Hz). The effect of torpor on sigma frequencies might be interpreted as an effect of the circadian system. In humans, sigma frequencies have a pronounced circadian modulation (5). In hibernating animals, the circadian system may be active, since the brain structure that contains the circadian pacemaker, the suprachiasmatic nuclei, is metabolically active (18). Moreover, it has been shown that body and brain temperatures continue to show circadian variation in hibernators (13, 21, 24, 35), and spontaneous arousal episodes are also often timed in a circadian fashion (6, 24). However, we induced all arousal episodes irrespective of circadian phase and observed the initial reduction in spectral activity of the sigma frequency range in all recordings. In addition, there were no detectable differences in spectral activity of the sigma frequency range between the EEG recordings following different sleep deprivation durations, i.e., in different circadian phases relative to arousal episode onset. Thus the reduction in spectral activity of the sigma frequency range appears not attributable to circadian variation and is apparently elicited by prior torpor.

Effects of torpor on EEG: due to a reduction in neuronal connections? The SWA enhancement by prior torpor, which is suppressed during sleep deprivation but not followed by a rebound after sleep deprivation, yields a conceptual problem. The enhancement cannot be explained by sleep regulation, since in this case sleep appears not to be a prerequisite for the reduction of SWA. On the basis of the available data, we propose a new concept to explain the reduction of SWA in the absence of sleep. We hypothesize that the initial enhancement of SWA is due to a general reduction of desynchronizing input on neurons, caused by a reduction of neuronal connections in torpor at low (below ~15°C) brain temperatures. A reduction in dendritic connections has been described for the hippocampus of torpid Arctic ground squirrels and appears to be restored early in the arousal episode (25, 26). Another indication is that thirteen-lined ground squirrels have a reduced density of pinealocyte synaptic ribbons in the hibernation phase (21). Also, synchronization of the NREM or REM sleep state of single neurons in the

hypothalamus of the golden-mantled ground squirrel is affected in the early stage of arousal episodes (19). If electrical activity is necessary not only to strengthen but also to maintain neuronal connections (15, 16, 29), this loss of connectivity could be explained by the largely reduced amount of firing of neurons below $\sim 15^{\circ}\text{C}$, described in vivo (19) and in vitro (23). At low brain temperatures, some neuronal activity remains present in the limbic system (35) and the hypothalamus, which has been interpreted as the persistence of a system that is able to thermoregulate and arouse in reaction to appropriate stimuli (14). The disappearance of electrical activity from different brain areas during reentry into torpor has a specific sequence, which is reversed during rewarming (14). Electrical activity in the cortex disappears first. Any remaining electrical activity in other brain areas apparently does not prevent the cortical EEG from being isoelectric at low brain temperatures and may be insufficient to keep the neuronal connections in the whole brain intact.

Loss of neuronal connections in torpor at low temperature would readily explain the reduction in the spindle or sigma frequency range after torpor in hibernators (31–34, 36), which is not observed after euthermic sleep deprivation and also not after daily torpor in Djungarian hamsters in which T_{crit} was above $\sim 18^{\circ}\text{C}$ (9–11). Spindle oscillations are dependent on neuronal “feedback” signals synchronizing electrical activity while hyperpolarization of neurons in the early NREM sleep stage is building up (30). In contrast, slow waves occur when neurons at more extreme hyperpolarization assume a burst-firing mode (30). A recent hypothesis on the mechanisms of sleep regulation suggests that energy depletion of neurons and glia during wakefulness would result in an increase in adenosine, which induces hyperpolarization of neurons and subsequently enhances SWA during NREM sleep (3). Indeed, the enhancement of SWA by wakefulness appears mediated specifically by the hyperpolarizing effects of adenosine (27). One may hypothesize that an influence that directly reduces depolarization of neurons may yield a similar effect. Reduced neuronal connectivity may reduce the depolarizing influences on neurons in the initial hours of arousal episodes. As a consequence, a spontaneously assumed burst firing mode may be maintained by this reduction of (disturbing) input.

Thus reduced connectivity could hypothetically result in an increase in SWA during NREM sleep after torpor, which has been consistently found but, as shown in the present experiments, cannot be attributed to “sleep-depriving” effects of torpor. Due to restored electrical activity of neurons when body and brain temperatures are high, neuronal connections would be regenerated in the initial hours of euthermy in arousal episodes, whether in sleep or in wakefulness, such that the drive to express SWA decreases independently of whether the animal sleeps or not.

In the hypothesis presented above, the increase in SWA following torpor of progressively longer duration (32) can be attributed to a progressive loss of neuronal connectivity in deep torpor. The hypothesis can also

explain why the initial high SWA is not observed after torpor at higher ambient temperatures (15°C , Ref. 31; $>15^{\circ}\text{C}$, Ref. 20), at which sufficient electrical activity may persist (19) to maintain neuronal connectivity. Functionally, the hypothesis would not attribute a primary function of the arousal episode to the regulation of sleep, as in our earlier hypothesis (7), but to the high brain temperature required to restore neuronal connections. This function is probably different from the situation in animals with daily torpor. The results of EEG analysis following daily torpor in Djungarian hamsters seem compatible with a sleep-depriving effect of torpor (9–11), although it has not been tested whether the enhanced SWA after daily torpor actually can be postponed by euthermic sleep deprivation. In daily torpor, T_b rarely drops below 12°C (12), whereas some hibernators track the ambient temperature down to subzero T_b (1, 31). Perhaps persistent foraging and other activities of animals with daily torpor require a fully functional brain, which can recover from the possible sleep-depriving effects of daily torpor and protect against loss of neuronal connectivity by maintaining a relatively high brain temperature during torpor. In hibernators such as ground squirrels, this may be of less importance because these animals typically remain sequestered in their burrows until spring. An indication that the brain of a hibernating animal is not functionally identical to the summer condition may be the disproportionately large reduction of sigma frequencies (7–14 Hz) in hibernating animals, which we observed in the EEG spectra of European ground squirrels, compared with the same animals having continuous euthermy during the following summer (Ref. 34 and A. M. Strijkstra and Q. van Katwijk, unpublished observations).

In conclusion, sleep during arousal episodes does not serve a function in reducing sleep-depriving effects of deep torpor in hibernating European ground squirrels. The initial SWA enhancement by torpor was not postponed when expression of SWA was suppressed by sleep deprivation, as would be predicted by normal sleep regulation processes. Thus it is unlikely that sleep homeostasis plays a role in the timing or the functional value of arousal episodes in hibernators, as we hypothesized earlier (7). Because the effects of deep torpor on the NREM sleep EEG appear dissimilar from sleep regulation, there must be another underlying process. The enhancement of SWA after deep torpor in hibernating European ground squirrels may indicate the recovery of the brain from a phase of electrical inactivity during deep torpor.

Perspectives. The neuronal connection hypothesis provides a possible explanation for some of the changes in the NREM sleep EEG that are observed after deep torpor. It does not, however, provide an explanation for the mechanism behind arousal episode occurrence nor does it directly indicate a functional value of arousal episodes. Further research is needed on finding the mechanisms of arousal episode occurrence. In addition, investigations into the changes in neuronal connections in torpor and during arousal episodes, in both the

mechanisms of change and the possible functional value, are needed. Arousal episodes have survival value because taking away the ability to arouse causes death (28). The process that causes death when arousal episodes are prevented is not known, but degeneration of neuronal connections may serve as a candidate.

We thank Roelof Hut, Ina Everts, and Peter Meerlo for their help in manually sleep depriving the animals. We thank Brian Barnes, Peter Meerlo, Tom Deboer, and Rudi van den Hoofdakker for their useful comments on the manuscript. We thank John Dittami and Eva Millesi for providing the animals.

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Received 14 April 1997; accepted in final form 8 July 1998.

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