Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated

Tom Deboer[CA] and Irene Tobler

Institute of Pharmacology and Toxicology, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland

[CA]Corresponding Author

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Animals emerging from hibernation or daily torpor show an initial increase in electroencephalogram slow-wave activity (SWA, power density between 0.75 and 4.0 Hz) in non-REM sleep, which subsequently declines. These typical features of sleep following prolonged waking led to the interpretation that the animals incur a sleep deprivation (SD) during torpor. This hypothesis has recently been questioned because the increase in SWA disappears in ground squirrels when sleep deprived immediately following hibernation. Here we show that in Djungarian hamsters subjected to SD immediately after daily torpor a predictable increase in SWA occurs during recovery. This supports the notion that the hamsters must sleep to dissipate the pressure for SWA incurred during torpor. The similarity between sleep after waking and torpor may provide a key for understanding sleep regulation. *NeuroReport* 11:881–885 © 2000 Lippincott Williams & Wilkins.

Key words: Daily torpor; Djungarian hamster; EEG; Hibernation; Sleep; Sleep deprivation; Spectral analysis

INTRODUCTION

It is generally accepted that hibernation and daily torpor developed out of sleep for energy conservation. Behaviorally and physiologically sleep, daily torpor and hibernation form a continuum [1]. However, after termination of the torpid state animals immediately begin to sleep in a similar way to that seen after sleep deprivation (SD) [2–6]. Thus, the power density in the slow-wave range of the electroencephalogram (EEG) in non-REM (NREM) sleep shows an initial increase and a subsequent gradual decline to baseline values in the following 2–3 h. EEG slow-wave activity (SWA; EEG power density 0.75–4.0 Hz) in NREM sleep is an indicator of NREM sleep intensity and is thought to reflect a sleep regulating process which keeps track of the prior history of sleep and wakefulness [7]. It was shown in the Djungarian hamster [8] and in hibernating ground squirrels [3,6] that the initial value of SWA is a function of torpor bout duration, similar as after wakefulness [9]. Thus, it seems that sleep during euthermic episodes in the hibernation season in ground squirrels or after daily torpor in the Djungarian hamster is a consequence of SD incurred during torpor [2,3].

Recently this hypothesis was rejected because the increase in SWA was absent when ground squirrels were subjected to SD immediately following deep torpor [10,11]. This lack of homeostatic regulation of SWA led to the conclusion that its increase is only related to the initial part of euthermia, and qualitatively different from SWA following prolonged waking. Moreover, when hibernation occurred at brain temperatures $>15°C$ it was not followed by an increase in SWA [6,12]. It was proposed that the low temperature during deep hibernation leads to a loss of neuronal connections which are restored during the high SWA in euthermia [10,11]. This hypothesis is supported by the reduction of dendritic connections in the hippocampus of arctic ground squirrels during deep torpor and their restoration during the subsequent euthermic periods [13,14]. The decrease in EEG power density in the 7–14 Hz band during NREM sleep in animals during the hibernation season compared to summer was also interpreted as being caused by a difference in brain connectivity [11].

If the initial post-torpor increase in SWA is related to the restoration of neuronal connections, then why does the Djungarian hamster, whose brain temperature remains $>20°C$ [4] show such an increase as well? To further investigate this question we sleep deprived hamsters after they emerged from torpor and compared the subsequent sleep EEG with the effects of torpor and SD alone.

MATERIALS AND METHODS

Animals: Adult male Djungarian hamsters (*Phodopus sungorus*), raised in summer under natural photoperiod, were kept in macrolon cages ($36 \times 20 \times 35$ cm) at 14.5°C ambient temperature (T_A, lights from 09.00–17.00 h, 60–130 lux) with food and water available *ad lib.* Animals were selected for implantation of EEG and electromyogram (EMG) electrodes when episodes of daily torpor were recognized and when the change in weight and fur color indicated a strong adaptation to the short photoperiod.

At the age of 5.4 ± 0.3 months the selected individuals (mean weight 26.4 ± 0.8 g, $n = 8$) were implanted with electrodes for EEG and EMG recordings and a thermistor to measure cortical temperature (T_{CRT}). The electrodes and the thermistor were soldered to a plug and attached to a cable that was fixed to the skull and anchored to three screws with dental cement [15]. For recording the animals were transferred to a sound-attenuated chamber where they were exposed to similar conditions as before (L:D 8:16 h; daylight-type fluorescent tubes, 18 W, 100 lux; 14.5°C T_A).

Experimental protocol: EEG, EMG and T_{CRT} were recorded continuously until torpor occurred. If torpor duration exceeded 4 h, and the animal started to warm up the torpor episode was randomly assigned to be either a control, followed by sleep (T), or to be followed by 1.5 h SD (T + SD). For T + SD the animal was awakened upon reaching 27–30° T_{CRT}. A day where no torpor occurred either served as baseline (BL), or the animal was submitted to 1.5 h SD at approximately the same time of day when previously torpor had terminated spontaneously. Never were torpor terminations induced by disturbing the animals. BL, T, SD and T + SD invariably occurred within the same week. SD was attained by tapping on the cage and by introducing objects (e.g. nesting material) into the cage whenever the animal appeared drowsy or assumed a sleeping position.

Data acquisition and analysis: The EEG and EMG signals were amplified (amplification factor ~2000), conditioned by analog filters (high-pass filter −3 dB at 0.016 Hz; low-pass filter −3 dB at 40 Hz; less than −35 dB at 128 Hz), sampled with 512 Hz, digitally filtered (EEG: low pass FIR filter 25 Hz; EMG: band-pass FIR filter 20–50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for consecutive 4 s epochs by FFT routine within the frequency range 0.25–25.0 Hz. Between 0.25 and 5.0 Hz the values were collapsed into 0.5 Hz bins, and between 5.25 and 25.0 Hz into 1 Hz bins. EMG signals were integrated over 4 s and T_{CRT} and T_A inside the cage were recorded at 4 s intervals. Before each recording the EEG and EMG channels were calibrated with a 10 Hz sine wave, 300 μV peak to peak signal. After the experiment the vigilance states were determined for 4 s epochs by visual scoring [15]. Epochs containing EEG artifacts were omitted from further analysis of the power spectra ($3.1 \pm 0.9\%$ (s.e.m.) of total recording time, $n = 8$), but vigilance states could always be determined. T_A inside the cage at the level of the animal was 14.5 ± 0.1°C and did not differ between the 4 recording days (ANOVA factor day, $p > 0.99$).

Brief awakenings were defined as short waking episodes (< 16 s), determined by an increase in EMG activity and a reduction of amplitude in the EEG. The number of brief awakenings (nBA) is a correlate of sleep intensity, inversely related to SWA in NREM sleep [16].

As previously under the conditions of short photoperiod and low T_A [4] EEG SWA in NREM sleep and the amount of the different vigilance states did not show a significant change with time of day over the 24 h baseline day ($p > 0.36$, one-way ANOVA over 1 h values). Therefore, the spectral data were calculated with the 8 h baseline light period as a reference.

Analysis of variance (ANOVA) for repeated measures served to determine overall differences between the 3 treatment days on the basis of hourly or 30 min values. Whenever significant effects were present ($p < 0.05$) further differences were evaluated by two-tailed paired *t*-tests.

Since the EEG power spectrum can be influenced by brain temperature [17,18], the effects of torpor and SD on the spectra were corrected for the temperature difference between the conditions. As described previously [18], a temperature coefficient (Q_{10}) value of 2.5 obtained in the same species [17] was applied to every frequency bin.

RESULTS

In the first hour of recovery, EEG power density in the slow-wave range during NREM sleep was invariably increased above baseline levels in all conditions (Fig. 1). Moreover, after T and T+SD power density in the slow-wave range was significantly above SD alone. After all three conditions the effect rapidly dissipated reaching baseline values after ~1–2 h. Power density in the frequency range 6–18 Hz was significantly higher in the first hour after T + SD and SD than after T alone (Fig. 1).

The SD was almost total and did not differ between T + SD and SD alone (98.0 ± 0.6 and 96.9 ± 1.1 waking as a percentage of recording time (mean ± s.e.m.); $n = 8$; $p > 0.4$ two-tailed paired *t*-test). Mean T_{CRT} did not differ significantly in the three 1 h intervals of recovery, but in the first hour T_{CRT} was ~1°C lower after T, compared with the other two conditions (Table 1). To compensate for this temperature difference between T + SD and T, the spectrum after T was corrected on the basis of the individual differences in T_{CRT} between T and T + SD, with a method described previously [18]. The resulting spectrum for T became almost identical to the one after T + SD (Fig. 2).

The increase in the slow-wave range was confirmed when the entire SWA band was computed (Fig. 3). SWA in NREM sleep was above SD alone in the first 30 min after T + SD, whereas after T it was intermediate and did not differ from either of the other conditions. SWA declined faster after SD and T + SD, reaching baseline levels after 1.5 h, while after T the decline was slower and the difference to baseline lasted for 2–2.5 h. No differences between the conditions were observed for the vigilance states, neither on the basis of 1 h (Table 1) nor 30 min intervals (data not shown). T_{CRT} differed significantly between all three conditions in the first 30 min of recovery (Fig. 3). nBA per hour of TST was significantly lower in the first hour after T + SD compared with SD alone, whereas after T nBA did not differ from either of the other conditions (Fig. 3).

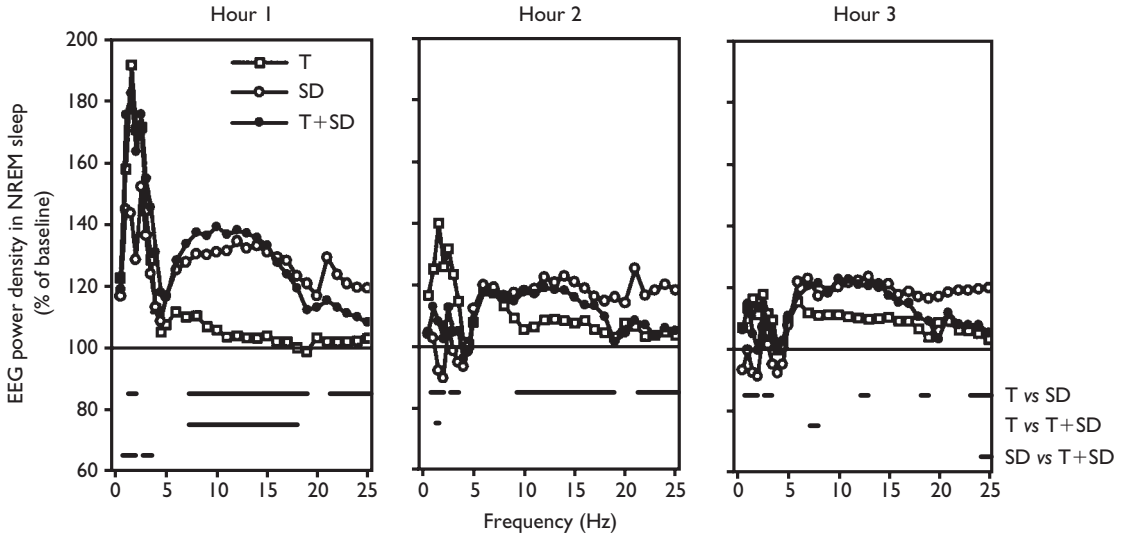

Fig. 1. EEG power density in NREM sleep in the first, second and third hour of recovery after torpor (T), sleep deprivation (SD), and torpor followed by sleep deprivation (T + SD). Curves connect mean values of relative power density for 1 h intervals ($n = 8$). For each frequency bin the data are expressed relative to the values of the baseline light period (= 100%). Values are plotted at the upper limit of each bin. Lines above the abscissa indicate frequency bands which differed between the conditions ($p < 0.05$, two-tailed paired *t*-test, after significant ANOVA for repeated measures).

Table 1. Vigilance states and cortical temperature (T_{CRT}) during NREM sleep in the first 3 h after the three conditions.

	Torpor	Sleep deprivation	Torpor + sleep deprivation
Waking			
Hours 1	17.9 (3.9)	24.7 (4.5)	22.5 (3.1)
2	25.2 (4.4)	37.2 (7.0)	25.7 (7.4)
3	29.5 (8.3)	46.0 (6.9)	35.7 (5.7)
NREM sleep			
1	72.9 (3.7)	67.5 (3.7)	71.2 (3.2)
2	66.6 (4.4)	53.5 (6.2)	59.7 (5.3)
3	62.0 (7.3)	47.7 (5.5)	54.5 (3.2)
REM sleep			
1	9.2 (3.1)	7.9 (1.6)	6.3 (1.1)
2	8.2 (2.1)	9.3 (1.6)	14.5 (3.2)
3	8.5 (2.7)	6.3 (1.8)	9.8 (1.0)
T_{CRT} in NREM sleep (°C)			
1	33.3 (0.6)	34.2 (0.5)	34.5 (0.5)
2	34.1 (0.6)	34.0 (0.5)	34.1 (0.5)
3	33.9 (0.6)	34.1 (0.6)	33.9 (0.5)

No significant differences were found between the three conditions.
Values are mean ± s.e.m. percentage of recording time ($n = 8$).

DISCUSSION

In the Djungarian hamster an additional SD after daily torpor led to a higher initial SWA than SD alone, indicating that the post-torpor SWA increase observed previously [4,8] is homeostatically regulated. The progressive decrease during subsequent sleep suggests a gradual recovery. Thus, SWA does not reflect a transient process related to the initial part of euthermia, and sleep is required for the decrease. Therefore, our results show that the initial SWA increase and its subsequent gradual decline are sleep related. Moreover, we show that nBA, another measure for sleep intensity, was markedly reduced after T + SD com-

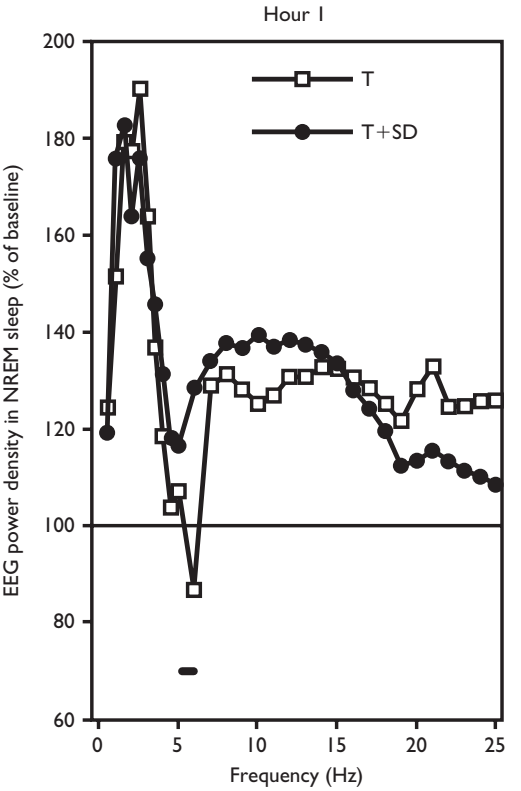

Fig. 2. EEG power density in NREM sleep in the first hour of recovery after torpor (T) and torpor followed by 1.5 h sleep deprivation (T + SD). The values after torpor are corrected for the individual temperature differences between the first hour after T and after T + SD (~1.2°C; method in [18]). Curves connect mean values of relative power density ($n = 8$). The data are expressed relative to the values of the baseline light period (= 100%). The line above the abscissa indicates the frequency bin (5.25–6.0 Hz) which differed between the conditions after correction ($p < 0.05$, two-tailed paired *t*-test).

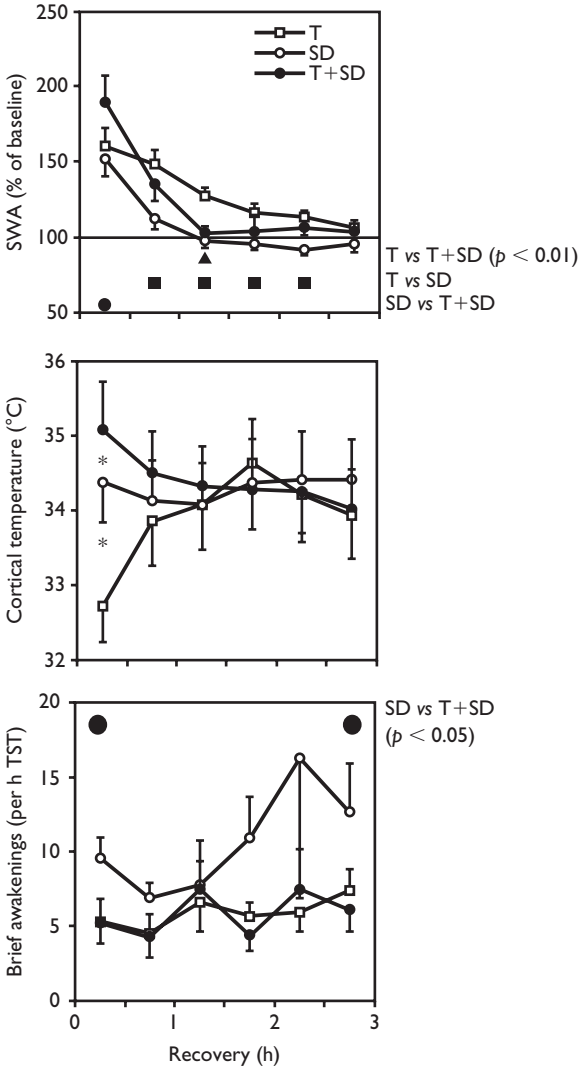

Fig. 3. Time course of mean values (± s.e.m.; $n = 8$) of slow-wave activity (SWA, mean EEG power density 0.75–4.0 Hz) in NREM sleep, cortical temperature (T_{CRT}), and number of brief awakenings, in the first 3 h of recovery after torpor (T), sleep deprivation (SD), and torpor followed by sleep deprivation (T + SD). SWA is expressed relative to the mean value over the 24 h baseline (= 100%). Brief awakenings are expressed/h of TST. Significant differences in SWA are indicated by symbols ($p < 0.01$, two-tailed paired *t*-test). For T_{CRT} all conditions differed significantly in the first 30 min interval ($p < 0.05$, two-tailed paired *t*-test). Brief awakenings differed significantly between SD and T + SD in the first and last 30 min interval of the 3 h ($p < 0.05$, two-tailed paired *t*-test, *t*-tests for all variables after significant ANOVA for repeated measures).

pared with SD alone. This result further supports the notion that daily torpor corresponds to prolonged waking.

After its initial increase SWA decreased more slowly after T than after SD and T + SD, particularly in the first 30 min. The speed of decrease in SWA is a function of the amount of NREM sleep [7], but since it was similar between the conditions it cannot be the cause of this difference. Other factors related to the SD (e.g. stress) may have influenced its time course. However, it is unknown whether stress or SD per se can influence the dynamics of the decrease of SWA. T_{CRT} differed between the conditions in the initial part of recovery. We have previously proposed that there is an optimal brain temperature for the generation of high amplitude slow waves or the sleep regulatory process underlying their generation [19]. T_{CRT} of animals emerging from torpor is further away from the proposed temperature optimum than after SD, and should lead to a slower SWA decrease rate in the initial part of recovery, which corresponds to our results (Fig. 3). The present data suggest that the lower brain temperature after T slows the sleep regulatory process, whereas the generation of slow waves is less affected.

A reduction in EEG power density in the 7–14 Hz range during NREM sleep has been reported for hibernators during euthermic periods between hibernation bouts in comparison to summer conditions [20]. This was interpreted as support for the notion that during euthermic periods the brain is functionally different from the brain during summer [11]. Our data suggest that also a lower brain temperature in winter can lead to the lower power density in this frequency range.

The different results for SWA in NREM sleep from ground squirrels and the Djungarian hamster could be caused by species specific differences. On the other hand, the possibility remains that the increase in SWA after torpor reflects a sleep-related process, but not sleep regulatory mechanisms. We consider this to be unlikely. In our experiment the amount of sleep during the SD was reduced to < 3%, a large reduction compared to the experiments in the hibernators where 13–20% sleep occurred despite the SD [10,11]. In the hibernators, the subsequent SWA did not exceed baseline levels. It is possible that a partial SD, suppressing SWA but allowing some sleep to occur, would lead to similar results. Further experiments are needed to resolve this issue.

Considering that torpor is a sleep-like state, what is the cause of the increase in SWA after torpor, which seems to be functionally similar to the increase after prolonged waking? One similarity between torpor and waking is the absence of high amplitude SWA. We have previously proposed that this absence during torpor may be similar to SD [17]. Another similarity is that during waking and at the end of torpor brain temperature increases rapidly. In brain slices a fast increase in temperature causes a depression in synaptic transmission which is mediated by an increase in adenosine release [21,22]. It was recently proposed that enhanced adenosine release in the brain eventually induces sleep and an increase in SWA [23]. Moreover, stimulation of adenosine receptors is followed by an increase in SWA [23,24], and SD may lead to a progressive adenosine increase [25]. An increase of adenosine release during arousal from torpor may be the consequence of an imbalance in energy metabolism of neurons, due to the fast increase in temperature. Such an imbalance has been proposed for brain slices [21]. However, it is unclear how to reconcile this interpretation with the significant positive correlation between torpor bout duration and subsequent SWA [7,8], and the lack of a significant correlation with the increase rate in brain temperature during arousal [8].

CONCLUSION

Our data show that the increase in SWA and its subsequent decline following daily torpor are related to NREM sleep and are homeostatically regulated. This supports the hypothesis that, despite behavioral and physiological differences in activity, metabolic rate, food intake, mean brain temperature and information processing between animals awake and those in daily torpor, the need for sleep increases during both states. It remains unclear what processes are responsible for the increase in SWA following both daily torpor and prolonged waking. It is reasonable to assume that the answer may lie in similar processes occurring during both states.

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