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Sleep during arousal episodes as a function of prior torpor duration in hibernating European ground squirrels

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SUMMARY   EEG's were recorded in hibernating European ground squirrels during euthermic arousal episodes at an ambient temperature of 5.5°C. Spontaneous torpor bouts ranged from 6 to 15 days, body temperature during torpor was 7.5°C. The torpor duration prior to EEG measurements was experimentally manipulated: the animals were induced to arouse by gentle handling after torpor of less then 1 day ($n=3$), 1–2 days ($n=6$), 3–4 days ($n=9$) and 5–12 days ($n=9$). The animals slept 71.5% of euthermic time, of which 61.4% NREM and 10.2% REM sleep. NREM percentage was slightly positively and REM percentage negatively correlated with prior torpor duration (TD). Spectral analysis showed changes in EEG activity during the euthermic phase in the slow wave frequency range (1–4 Hz) and in higher frequencies. Prior TD specifically affected the slow waves. Slow wave activity decreased exponentially during the euthermic phase. The initial slow wave activity showed a systematic increase with prior TD, which could be described by an exponentially saturating function, albeit with a relatively small time constant compared with spontaneous torpor duration. It is concluded that sleep during arousal episodes following torpor at an ambient temperature of 5.5°C is affected both in structure and intensity by prior TD. The results are consistent with the proposition that torpor inhibits the restorative function of sleep.

KEYWORDS   ground squirrel, hibernation, periodic arousal episode, sleep, spectral analysis, torpor

INTRODUCTION

In hibernation mammals minimize energy expenditure in a state of torpor. This enables them to survive extended periods of reduced food supply. During torpor metabolic rate in these animals can be 1% of the euthermic minimal metabolic rate, depending on the temperature gradient they sustain during torpor (Heldmaier and Ruf 1992). All hibernators interrupt this torpor state regularly to return to euthermy. These euthermic phases are called 'arousal episodes' and last 4–24 h, depending on species and size (French 1985). Arousals are expensive in terms of energy (Wang 1979; Kenagy *et al.* 1989). The physiological function of arousal episodes is not known. Several theories have been put forward, but there is no conclusive experimental evidence (Willis 1982).

Recently, a new theory on the function of arousal episodes has been proposed (Daan *et al.* 1991; Trachsel *et al.* 1991). In two species of ground squirrels EEG measurements during the euthermic phase of arousal episodes suggested that a sleep debt accumulates during torpor. Within the euthermic phase slow wave activity, an indicator of both sleep debt and sleep debt decrease in NREM sleep (Borbély and Neuhaus 1979; Neckelmann & Ursin 1993), gradually decreased. This suggests that arousal episodes may be necessary to facilitate sleep processes. Both studies proposed that at low body temperatures sleep and thereby some of its intrinsic physiological functions are suppressed, and sleep debt accumulates. Trachsel *et al.* (1991) and Daan *et al.* (1992) found an increase of slow wave activity with prior TD. Trachsel *et al.* (1991) compared sleep EEG measurements during arousal episodes in the Golden-mantled ground squirrel (*Spermophilus lateralis*) after spontaneously terminated torpor bouts vs. artificially terminated (shortened) TDs. In this comparison the effects might also be attributable to differences between spontaneous

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and induced onset of an arousal episode. Daan *et al.* (1992) compared EEG measurements in the Arctic ground squirrel (*Spermophilus parryi*) during artificially induced arousal episodes after different TDs. In that study it was unknown how sleep variables behave during a complete euthermic phase, since only the first 6 h of the euthermic phase were considered. In the present study we recorded EEG's during complete euthermic phases in the European ground squirrel, *Spermophilus citellus,* and analysed these over the euthermic phase of the arousal episode. All arousal episodes were artificially induced after different TDs.

METHODS

Nine adult European ground squirrels (*Spermophilus citellus*) were used, four males (pre-hibernation body mass: 397 g (s.e.m. 23)) and five females (pre-hibernation body mass: 281 g (s.e.m. 11)). Measurements were made in two hibernation seasons (1991–93). Prior to hibernation, animals were housed individually in $60 \times 40 \times 40$ (high) cm steel cages (sawdust bedding, 20°C, light/dark cycle 12L:12D). Hay was provided as nesting material. Animals were fed *ad libitum* with commercial rabbit food pellets (Teurlings).

In late September, EEG and EMG electrodes were implanted under pentobarbital anaesthesia ($60 \, \text{mg kg}^{-1}$). Silver EEG electrodes were placed on the parietal cortex and on the cerebellum. Stainless steel EMG electrodes were placed under the skin on the neck muscles (MS 303, Plastic One inc., Roanoke, Virginia). Two stainless steel screws placed bilaterally through the skull on the frontal cortex served as a ground. A calibrated thermistor sealed in a glass capillary (1.0 mm in diameter) was placed on the frontal cortex. All electrodes were fixed to a connector attached to the two frontal screws, and an additional one placed above the parietal cortex with dental cement.

In early November the animals were put in continuous dim light (less than 1 Lux) at 5.5°C to allow hibernation. All animals had entered hibernation by early December. Animals were checked daily by visual inspection or touch. Arousal episodes were scored when animals were warm and active when touched, or when the position in the nest had changed since the previous day.

EEG recordings were made from early December to early March, during which phase of hibernation spontaneous torpor bout length is relatively constant in most hibernators (French 1985; Geiser and Kenagy 1988). Recordings were made in the animal's home cage. Animals were induced to arouse by gentle handling after various TDs. The animals were placed at room temperature and handled until they showed voluntary movements when laid down on a flat surface. After induction of the arousal episode, animals were connected to an EEG/EMG amplifier (Twente Technology Transfer: EEG: Sensitivity 200 µV/V, Frequency range 0.2–80 Hz, CMRR >100dB; EMG: Sensitivity 500 µV/V, Frequency range 20–600 Hz, CMRR >100dB) with a seven-strand flatcable via a slip ring swivel (Air Precision, Le Plessis Robinson, France). The amplifier was connected to an AD converter (PCLabcard 812, Advantech, The Netherlands) in a PC-AT286. Data were acquired and processed by the EEG recording and analysis program POLY (vs.4.70, Inspector Research Systems, Amsterdam, The Netherlands). The signal was filtered by a software filter (-3 dB at 17 Hz, 35 dB per octave), sampled at 100 Hz, and analysed on line by Fast Fourier Transformation per 10-s interval, and stored at 20 Hz for later visual scoring for wakefulness (WAKE) and NREM and REM sleep stages. After Fast Fourier Transformation, average EEG power was calculated per 2-h interval for 0.2–15 Hz in 1 Hz bins. Power values were normalized to correct for interindividual differences in EEG signals, yielding relative activity values. This was carried out per Hz for the complete spectrum (frequency activity 0–15 Hz), and for the slow wave (1–4 Hz) region of the spectrum alone (slow wave activity, SWA). Several technical problems were encountered with the assessment of cortical temperature. For three animals in 1–3 recordings the cortical temperature could be used for analysis.

Recordings were made after various TDs, for comparison they were categorized according to the number of days torpid preceding the recording. Six animals were recorded during arousal episodes after TDs of 1–2 days, 3–4 days and 5–12 days. Three animals were recorded after a TD of less than 1 day, 3–4 days and 5–12 days. Rectal temperature was measured immediately after the animal was picked up for arousal episode induction, except for the measurements after a TD of less than 1 day. Since the latter recordings were made as continuations of a previous EEG measurement, the TD could be more precisely estimated from these recordings and were expressed in hours. TDs of 1 day or more were based on the daily check on arousal episode occurrence and were expressed in days.

An arousal episode consists of a warming up phase and an extended euthermic phase, which ends when the animals reduce their metabolism and start cooling down. In order to avoid potential effects of temperature on the EEG variables in the analysis, we excluded data before or after the euthermic phase. We operationally defined the start of the euthermic phase as the time when the high EMG surge observed at the early stage of the arousal episode ceased, indicating the end of shivering thermogenesis. The end of euthermy was defined as the start of the last substantial activity bout before the sleep bout during which EEG power clearly declined with the entry into torpor. In Fig.1 the cortical temperature, EMG activity and total sleep percentage (NREM + REM) is shown from 1 h before to 1 h after onset and offset of the euthermic phase following these definitions for three individuals. Cortical temperatures above the lowest cortical temperature during a 2 min continuous wake interval within the euthermic phase (30.8°C (s.e.m. 1.0)) were reached 12.4 min (s.e.m. 1.0) after the cease of the initial high EMG activity. Excluding EEG delta power during this 12.4 min interval, from the 2 h average delta power, resulted in a minor (2.1% (s.e.m. 6.2)) increase. Standard deviation of the cortical temperature over all 2 min intervals during the euthermic phase ranged from 0.3–1.1°C, indicating the relative stability of cortical temperature within our definitions of the
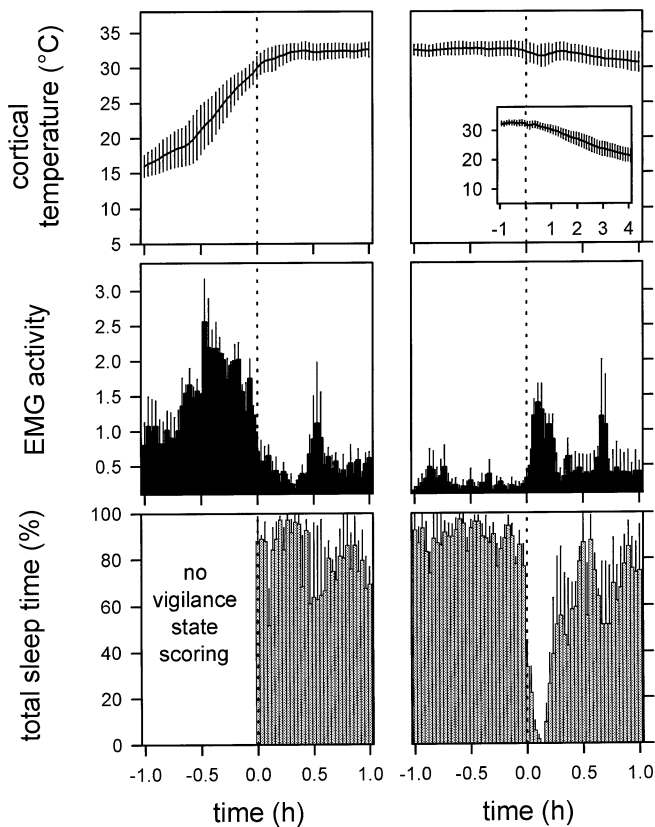
Figure 1. Cortical temperature, and concurrent EMG activity and sleep time (%) per 2-min interval (mean and s.e.m.), from 1 h before to 1 h after the borders of the euthermic phase. Time 0 indicates the onset (left panels) and offset (right panels). The upper panels depict the rise in cortical temperature prior to euthermy (left panel), and the slow decrease in cortical temperature when reentering torpor. The graph in the right panel depicts a larger time base (1 h before to 4 h after offset) to better indicate the decrease. The middle left panel indicates the high EMG activity related to shivering thermogenesis before time 0. Other high EMG activity was related to wakefulness. The lower panels depict total (NREM + REM) sleep percentage, indicating high amounts of sleep just after onset and prior to offset of the euthermic phase. Before the onset no vigilance states were scored. After the offset vigilance states were recognizable for some hours, but these data were not used in analyses.

euthermic phase. Within the euthermic phase the average cortical temperature during wakefulness was lowest (32.3°C (s.e.m. 1.3)), probably because when the animal is active, the head is more exposed to the low ambient temperature. In 2-min intervals during NREM sleep the cortical temperature was slightly lower (32.9°C (s.e.m. 0.7)) compared with REM (33.1°C (s.e.m. 0.7)). 0.4 Days after the end of the previous euthermic phase, the cortical temperature declined during the recooling phase to 13.6°C (s.e.m. 1.3). This value served as a temperature reference for the recordings after TDs of 0.4 days. Note that the cortical temperature sensors were placed on the cortex and exposed to a low ambient temperature. They can only be used as a relative measure of brain temperature and its changes.

Kruskal–Wallis non-parametric ANOVA (KWNPA) was used to test for differences between categories. Multiple

regression analysis and Spearman rank correlation (SRC) were used for investigation of dynamic effects.

RESULTS

The individual spontaneous torpor bout lengths during mid-hibernation ranged from 6 to 15 days and the means of the individuals averaged 10.6 days (s.d. 3.2, $n=9$). The two groups of animals receiving different prior TDs in the experiment had average torpor bouts of 11.0 (s.d. 3.5, $n=3$) days and 10.3 (s.d. 3.2, $n=6$). These were not significantly different. Rectal temperatures after TDs of 1 day and longer were 7.5°C (s.d. 0.75, $n=9$), and did not vary significantly between TD categories.

Our manipulated TDs ranged from 0.38–12 days, which were grouped in four categories with different average TDs. Average TD and the duration of the euthermic phase of the following induced arousal episode and the vigilance state percentages are shown in Table 1. Euthermic phase duration ranged from 3.9–22.3 h and averaged 13.6 (s.d. 5.9, $n=9$) h. Euthermic phase duration showed considerable variation with a tendency to be longer after longer TD.

Overall, animals slept for 71.5% of euthermic time during the arousal episodes, of which 61.4% was NREM and 10.2% REM sleep. Further analysis was confined to the first 8 h of euthermic time, because the number of animals contributing to the data rapidly decreased thereafter. Over the first 8 h total sleep time (NREM + REM) was on average 71.3% (s.d. 4.8, $n=9$) and did not show a correlation with prior TD. NREM sleep time was positively correlated (SRC: $r^2=0.41$, $n=27$, $P<0.05$), whereas REM sleep time was negatively correlated with prior TD (SRC: $r^2=-0.39$, $n=27$, $P<0.05$). When analysed with multiple regression on 2-h bins, the amount of NREM was positively associated with prior TD, and negatively related to time in euthermy (TE)(TD: $F_{1,100}=7.1$, $P<0.005$; TE: $F_{1,100}=21.7$, $P<0.0001$; $r^2=0.26$, $n=102$). The amount of REM sleep was negatively related with prior TD in interaction with TE (TD: $F_{1,99}=13.3$, $P<0.0005$; TE: $F_{1,99}=9.9$, $P<0.005$; TD*TE: $F_{1,99}=7.0$, $P<0.01$; $r^2=0.52$, $n=102$). After long TDs REM sleep was reduced to a greater extent during the first 4 h in euthermy (see Table 1 for test results, analysed per 2-h interval).

Figure 2 presents an example of EEG delta power dynamics in the raw data of one individual during the euthermic phase of arousal episodes after TDs of 9 h, 3 days and 6 days. Spontaneous torpor bout length was on average 9.3 days for this individual. The time course of EEG intensity of the slow wave frequencies (1–4 Hz) and concurrent EMG power are shown with a 1 min resolution. Initial high EMG values before time 0 indicate shivering thermogenesis. After the cease of these high EMG values, EEG delta power increased, indicating the occurrence of NREM sleep. The EEG delta power during the first sleep bout of ∼3–6 h was higher after a longer TD. After 3 and 6 days of torpor initial delta power values were high compared with the end of euthermic time. The arbitrary

Table 1 Euthermic phase duration and percentages of NREM and REM sleep during arousal episodes. Induced prior torpor duration (TD, days) and euthermic phase duration (EPD, hours) are shown as TD category averages (standard deviations), and *P*-values for test results (KWNPA) on variation between TD categories. The lower part gives the average percentage of NREM and REM sleep and the number of animals contributing to the average (*n*) per 2-h interval

| Category														
	TD<1 day			*TD 1–2 days*			*TD 3–4 days*			*TD 5–12 days*			*P*	
TD	0.4 (0.05)			1.3 (0.50)			3.5 (0.50)			7.6 (2.2)				
EPD	10.6 (8.3)			14.2 (9.9)			12.1 (3.4)			14.0 (4.9)			*NS*	
Time	NREM	REM	*n*	NREM	REM	*n*	NREM	REM	*n*	NREM	REM	*n*	NREM	REM
0-2h	67.5	10.0	3	67.2	6.3	6	73.7	1.5	9	71.3	0.6	9	NS	<0.001
2-4h	49.4	8.9	2	56.4	12.2	6	66.8	10.1	9	64.0	5.9	9	NS	<0.01
4-6h	58.0	11.6	2	50.1	11.8	5	52.9	11.8	9	68.5	14.1	9	<0.05	NS
6-8h	57.9	11.5	2	55.1	14.3	4	53.4	12.6	9	55.2	11.8	9	NS	NS
8-10h				60.9	14.7	4	60.7	12.5	7	66.5	17.6	8	NS	NS
10-12h				50.2	9.5	3	40.3	9.4	4	60.3	14.1	3	NS	NS
12-14h				45.1	9.4	2	60.0	12.9	3	59.9	11.3	3	NS	NS
14-16h				60.1	8.3	2	62.0	10.7	2	51.6	10.5	2	NS	NS
16-18h				34.6	5.2	2				67.9	15.6	2	NS	NS
18-20h				50.0	6.9	2								
20-22h				60.8	11.9	2								
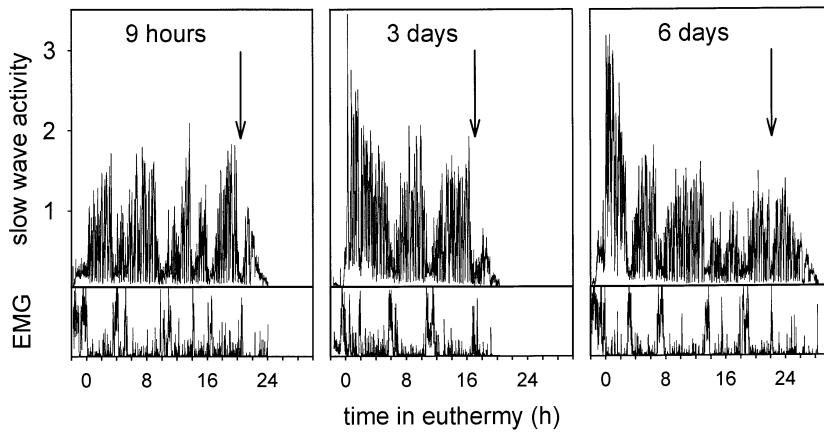
Figure 2. Normalized power of the slow wave frequency range (1–4 Hz, slow wave activity) of the EEG and EMG power throughout arousal episodes. Data are shown as 1 min averages of the raw data. The animal was induced to arouse after 9 h, 3 days and 6 days of torpor. Time 0 was defined as the end of the initial EMG surge, indicating the end of shivering thermogenesis. Arrows indicate the time after which data were not used in analyses to avoid temperature effects on the EEG. During the arousal the animal slept in bouts of 3–5 h indicated by high EEG power, with intermittent wakefulness indicated by high EMG power. Within a sleep bout regular REM sleep bouts occurred, dependent on TE and prior TD (see Table 1).
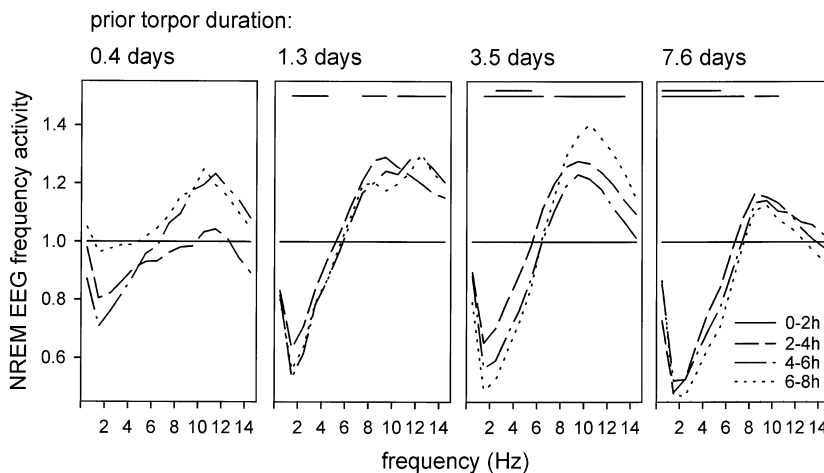

Figure 3. Relative spectral power (frequency activity) of the EEG during the euthermic phase, induced after various TDs. Data are shown per 1 Hz bin for 0.2–15 Hz in 4 panels with different average prior TD. Data are normalized relative to the power within that frequency bin during the first 2 h of euthermy, indicated by the horizontal line at frequency activity 1.0. The following 2-h intervals are shown by progressive dashing. Two levels of significance of variation between the four 2-h interval are indicated by horizontal lines at the top of the panels (1 line: *P*<0.05, 2 lines: *P*<0.001, KWNPA).

cut-off point of euthermy at the end of the arousal episodes is indicated by arrows.

Figure 3 shows the time course of frequency activity, e.g. relative power of the NREM EEG spectrum, for 0.2 Hz to 15 Hz in 1 Hz bins. For each TD category, wave activity is expressed relative to the frequency's power during the first 2 h of euthermy. Data for the first four 2-h intervals are shown. In the TD category of 0.4 days there was no significant difference in any frequency between consecutive 2-h intervals. The small sample size ($n = 3$) does not allow firm conclusions about the absence of effects. The size and direction of changes are in line with the changes in the other three categories. After longer TD, significant increases with TE were found in the frequencies above 8 Hz, and significantly decreasing effects in the frequencies from 1 to 7 Hz, including the slow wave range (1–4 Hz). Changes in power were tested as a function of log (TD) and log (TE) with a multiple regression analysis on log-transformed EEG power data per 1 Hz frequency bin. Significantly positive relations with log (TD) were found in the 1–8 Hz range. Both significant negative and positive relations were found as a function of log (TE): negative relations in the 1–6 Hz range, and positive relations in the 8–14Hz range. This indicates that power in the higher frequencies (8–14 Hz) is relatively independent of prior TD, but shows a temporal change over the euthermic phase. Changes in the lower frequencies increase in magnitude with increasing TD. Differences between categories in the lower frequencies were found in the first (KWNPA: $P<0.01$) and second 2-h interval ($P<0.05$).

The time course of slow wave activity in NREM sleep is shown in Fig. 4. The power of the slow waves for a given individual was expressed relative to the average slow wave power value in NREM over the first 8 h of the EEG recording after a prior TD of 3–4 days. Significant differences between groups were found for the first three 2-h intervals. Multiple regression analysis of log (SWA) in the first 8 h of the euthermic phase showed significant effects of TE ($F_{1,100}=42.9$, $P<0.0001$) and of prior TD ($F_{1,100}=14.6$, $P<0.0005$), and no significant interaction between the variables ($F_{1,99}=1.0$, $P=0.32$). This indicates that the rate of decrease of slow wave activity with TE (–0.042 log units per hour) is not statistically different for the TD categories, whereas the amount of slow wave activity is influenced by prior TD (0.018 log units per day of torpor).

In Fig. 5 the slow wave activity of the first 2-h interval is shown for all EEG recordings as a function of prior TD. A significant positive association was found (SRC: $r^2=0.64$, $n=27$, $P<0.002$). A model describing an exponentially saturating curve between a minimum and a maximum level was fitted, yielding the following equation:

$$SWA = 1.7 - 1.3 * e^{-prior\ TD/1.4}.$$

This model significantly enhanced the explained variance compared with linear regression ($F_{2,26}=8.1$, $P=0.0087$). This provides some evidence that the increase in slow wave activity levels off with progressively increasing TD.
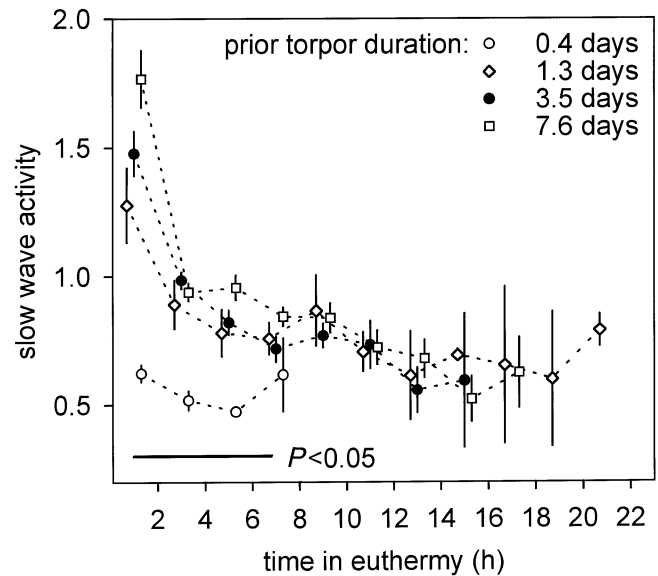

Figure 4. Time course of slow wave activity (mean and s.e.m.) during the euthermic phase of arousal episodes induced after various prior TDs. Slow wave power was normalized relative to the average value over the first 8 h of the arousal episode induced after 3–4 days of torpor (which is indicated by solid circles). Three other categories are shown with average prior TD of 0.4 days: open circles, 1.3 days: open diamonds, and 7.6 days: open squares. The number of animals contributing in the average values decrease in time and are stated in Table 1. Significant variation between categories was found in the first 6 h including the prior TD of 0.4 days category, and in the first 2 h excluding this category ($P<0.05$, KWNPA).
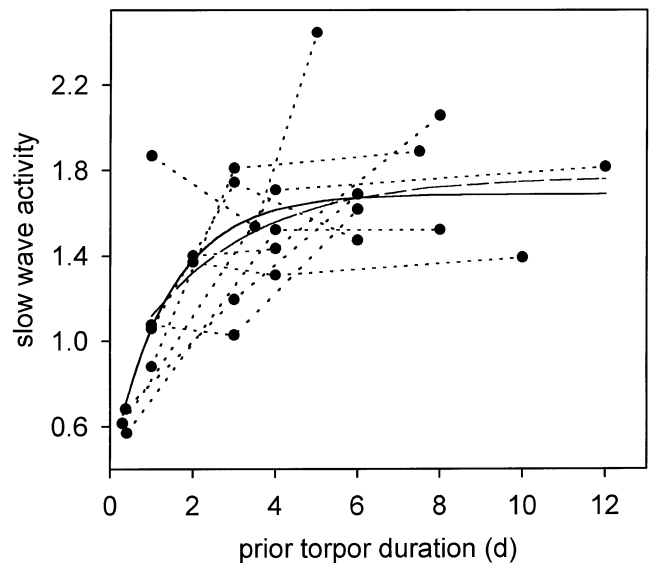

Figure 5. Slow wave activity during the first 2 h of the euthermic phase as a function of prior TD. Values of individual animals are connected by dotted lines. The curves indicate fitted exponentially saturating functions including (solid line: SWA $=1.7-1.3*e^{-TD/1.4}$) and excluding the less than 1 day prior TD data (SWA $=1.8-1.0*e^{-TD/2.7}$).

We realize that the arousal episodes after <1 day TD started at cortical temperatures of 13.6°C, well above the average 7.5°C body temperature after longer TD. Since temperature during torpor may have profound effects on subsequent sleep in the arousal episode (Larkin and Heller 1996; Strijkstra and Daan in press), we also analysed the data excluding the TD<1 day category. A positive association was found (SRC: $r^2 = 0.49$, $P<0.02$). This demonstrates that the rise in SWA with longer prior TD can not be attributed to a temperature effect alone. Fitting a saturating curve to these data only yielded the equation:

$$\text{SWA} = 1.8 - 1.0 * e^{-\text{prior TD}/2.7} \quad \text{(see Fig.5)}.$$

DISCUSSION

As in Golden-mantled (Trachsel *et al.* 1991) and Arctic ground squirrels (Daan *et al.* 1991), European ground squirrels spend most of the euthermic time during arousal episodes in sleep. Prior torpor duration (TD) affects the sleep in structure and intensity variables. The time spent in NREM sleep increased with prior TD, and time spent in REM sleep decreased. Total sleep time was not affected.

By comparing different prior TDs we obtained information on the effect of torpor on the dynamics of the EEG. EEG activity changes dependent on prior TD occurred mainly in the low frequencies 0.2–7 Hz, which includes the slow wave range. This may suggest that NREM sleep debt is specifically influenced by prior TD. Higher frequencies were affected only by the time elapsed in the euthermic phase, not by prior TD. The frequencies above 7 Hz showed a consistent change over the euthermic phase in all categories, independent of prior TD. These frequencies show a relative increase, at least from the first to the later 2-h intervals, suggesting that the generation of the waves in the spindle frequency range is impaired during torpor. These data closely resemble the spectral changes found by Trachsel *et al.* (1991), who found significant variation for 5–10 Hz. In those recordings the EEG's were measured during spontaneous arousal episodes. Thus, the pattern of changes in the EEG during arousal episodes does not appear dependent on the way of initiation of the arousal episode.

The difference between the category with a prior TD of 0.4 days and the other categories suggests that after a very short torpor phase, when the animals are still relatively warm, sleep debt is not yet increased. It also indicates that rewarming of the animal from a cortical temperature level estimated to be 13.6°C, does not have an elevating effect on slow wave activity.

Trachsel *et al.* (1991), Kilduff *et al.* (1993) and Berger (1993) have suggested that the initial high values of slow wave activity after arousing from torpor may be as a result of the metabolic effort of the preceding (shivering) thermogenesis. This hypothesis would predict that there is no dependence of slow wave activity on prior TD. Since in the present experiment all arousal episodes were induced artificially the effects are not attributable to differences in the way the arousal episode was

started. This finding, and the suggestion that rewarming from a cortical temperature of 13.6°C does not elevate slow wave activity mentioned previously, support the alternative interpretation preferred by Trachsel *et al.* (1991) of the increase in slow wave activity with longer TD as a result of the prior torpor and not to the warming up.

In a critique of the findings of Daan *et al.* (1991) and Trachsel *et al.* (1991), Berger (1993) commented on several possible shortcomings in the theory and these data. Berger (1993) suggested that the decrease in slow wave activity over an arousal episode is a result of a change in temperature of the animal. We excluded possible temperature effects at the end of the arousal episode by restricting the analysis to data before the last major wake episode. This is well before the cortical temperature slowly decreased (Fig.1). Furthermore, the cortical temperature traces never showed large fluctuations during euthermy, as also seen in the data from Trachsel *et al.* (1991). Hence, large effects on slow wave activity are unlikely to be because of temperature changes, since the animals usually have a stable body and brain temperature during the euthermic phase. The body temperature decrease in the figure (Daan *et al.* 1991: Fig. 2) which Berger (1993) refers to, was probably as a result of a position change of the fur temperature sensor. The decrease rate was much faster then the passive cooling rate (indicated further on in the same figure). If this sudden change reflected body temperature the animal should have been actively cooling, for which there is no precedent. Berger (1993) further commented on 'inconsistent' effects within the low frequency range which he defined as 0.25–7 Hz. The present data closely match the effects in the slow wave frequencies found by Trachsel *et al.* (1991), and do not show these inconsistencies. In addition, Berger (1993) raised some general sleep research issues concerning the possible recuperative function of sleep and slow wave activity as a potential indicator of the state of recuperation. It is now commonly accepted that slow wave activity in NREM sleep is regulated in some way as a function of prior wakefulness or sleep deprivation (Borbély and Neuhaus 1979; Zepelin 1989). This is supported in a behavioural sense, by the correlation of slow wave activity with responsiveness (Neckelmann and Ursin 1993).

Deboer and Tobler (1994) reported that after daily torpor in the Djungarian hamster (*Phodopus sungorus*) a sleep rebound occurred, resembling sleep deprivation effects both in quantity and spectral changes. Whether prolonged deep torpor in a hibernator also quantitatively resembles sleep deprivation effects on EEG in the non-hibernation state is currently under investigation. In comparison with the data on the slow wave activity rebound in *Phodopus,* which occur after torpor bouts of 3.8–6 h (Deboer & Tobler 1994), the effects in this report occur after considerably longer TD.

The decrease in slow wave activity during sleep within the euthermic phase could be described by an exponential decay function which did not show interaction with prior TD: the rate of decrease was not dependent on TD and TE, and the level was apparently set by prior TD. The overall rate of

decrease in slow wave activity was estimated to be 0.042 log units per hour. This is high compared with measurements of the Arctic ground squirrel (log (SWA) = 0.050–0.011*log (TE) (Daan *et al.* 1991)). The differences between the European ground squirrel (∼300 g), the smaller Djungarian hamster (∼30 g) and the larger Arctic ground squirrel (∼800 g) indicated above could be because of size related differences in the rate of sleep debt increase during torpor and decrease during arousal episodes.

The rate of sleep debt increase

The initial slow wave activity showed on average a monotonic increase with prior TD. This increase was significantly better fitted by a saturating curve than a linear regression, indicating that a limit exists for the maximal sleep debt which is reached during torpor. The estimate of the time constant (1.4 days) appears very short compared with the spontaneous torpor bout duration of 10.6 days: that is, under the assumption that sleep regulation of a torpor/arousal cycle is similar to sleep regulation during continuous euthermy. The model depends heavily on the data for the short TDs. Without these data, a positive association was still found: fitting the saturating model increased the time constant to 2.7 days. Note that in this case the saturating model did not fit the data better than a linear regression, and in fact we do not have a confirmed reason to assume that an asymptotic value is reached during torpor. Thus, we have evidence for two effects: (1) an increase in SWA with prior TD and (2) a suppression of this SWA increase after very short TD. Effect (1) can not be ascribed to acute differences in body temperature, since there were none. Effect (2) might be part of the same phenomenon, or might alternatively be attributed to the higher cortical and body temperature during and/or after very short TDs. If the latter is correct, the time constant of 1.4 days is irrelevant. One might surmise that either sleep debt accumulation in torpor is reduced at higher temperatures or that the initiation of the arousal process from a higher temperature reduces SWA. These data do not allow us to distinguish between these alternatives. Studies on spontaneous arousal episodes in the Golden-mantled ground squirrel (Larkin and Heller 1996) and on induced arousal episodes, controlling for differences in spontaneous TD in the European ground squirrel (Strijkstra and Daan in press) suggest that temperature during torpor plays an important role in the determination of the initial level of SWA in subsequent arousal episodes. Experimental manipulation of temperature during torpor could solve this issue, but this remains to be carried out. Given the uncertainty on the time constant of the TD dependence of SWA, there is not yet sufficient evidence to assess whether the process is a potential candidate for triggering arousal episodes as hypothesized by Trachsel *et al.* (1991) and Daan *et al.* (1991).

In conclusion, the present data indicate that a sleep debt accumulates during torpor, and hence torpor and sleep may be functionally dissimilar. This contrasts to propositions by Walker *et al.* (1979; 1981) and others that sleep and torpor are homologous processes. However, it remains to be shown that the arousal episode/torpor cycle is regulated by mechanisms linked to the sleep debt increase.

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