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Ambient temperature during torpor affects NREM sleep EEG during arousal episodes in hibernating European ground squirrels

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Abstract

Ambient temperature (T_a) systematically affects the frequency of arousal episodes in mammalian hibernation. This variation might hypothetically be attributed to temperature effects on the rate of sleep debt increase in torpor. We studied this rate by recording sleep electroencephalogram (EEG) in arousal episodes induced after 4 days of torpor at different T_a . Spontaneous torpor bout duration (TBD) varied with T_a : TBD was maximal at 5.5°C (10.7 days), and was reduced at lower (−5°C: 5.0 days, 0°C: 9.3 days) and higher T_a (10°C: 8.7 days, 15°C: 5.0 days). Slow wave activity (SWA) during non-rapid-eye-movement (NREM) sleep, an indicator for sleep debt, showed initial high values after torpor at T_a ranging from −5°C to 10°C. When torpid at 15°C, SWA was not increased in the subsequent arousal episode. The data are thus inconsistent with a rate of sleep debt explanation for the temperature dependence of TBD. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Sleep; Hibernation, Torpor; Arousal episode; Ambient temperature; Spectral analysis

Mammalian hibernation is not continuous. During hibernation mammals periodically interrupt torpor to return to euthermy in ‘arousal episodes’. Why and how the animals regulate the arousal episodes has received a fair amount of attention, but no satisfactory general answer is available [12]. The periodic nature of the occurrence and the restricted external cues that hibernating mammals are exposed to, suggests that an internal signal triggers arousal.

The hypothesis that arousal episodes are used for sleep has been addressed in a series of papers. Three predictions from this ‘arousal for sleep’ hypothesis have been supported by empirical results: (1) animals sleep most of the time [2,10], (2) a sleep debt, reflected by the non-rapid-eye-movement (NREM) sleep electroencephalogram (EEG) slow wave power, decreases during the arousal episode [2,10], and (3) there is evidence that sleep debt increases with duration of torpor [3,5,9,10]. It is known for several hibernators that spontaneous arousal episodes occur more frequently when ambient temperature (T_a) is

higher [11]. This may be interpreted in terms of the ‘arousal for sleep’ hypothesis in two ways: either the sleep debt builds up faster in torpor at higher T_a , or the threshold at which sleep debt triggers arousal is reduced.

The effects of T_a on NREM EEG during arousal episodes has been studied in the Golden-mantled ground squirrel by Larkin and Heller [8]. These authors measured EEGs during spontaneous arousal episodes and intermittent torpor at different T_a . They found a lower sleep debt during arousal episodes at higher T_a , following the shortest torpor bouts. Moreover, their data indicate an absence of sleep debt after torpor at high T_a [8]. This can be seen as a major problem for the ‘arousal for sleep’ theory, since arousals appear to occur even when sleep debt does not increase during torpor. The use of spontaneous arousals limits the information on the dynamics of the sleep debt during torpor: the sleep debt change may have occurred instantly by factors linked to the onset of the arousal episode. Additionally, there may have been influences of T_a during the EEG recording itself. In the present study, we avoided these problems by allowing the animals to be torpid at different T_a for 4 days, and record the EEGs always at the same T_a (5.5°C). We included sub-zero T_a ,

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since spontaneous torpor bout duration is also reduced at sub-zero T_a [6].

The experiments were carried out with 16 European ground squirrels (*Spermophilus citellus*) over the hibernation seasons of 1991–1992 and 1992–1993. Animals were housed individually in $40 \times 60 \times 40$ cm steel cages at L/D 12:12 h and 20°C with sawdust bedding and food and water ad lib. Early October, EEG and electromyogram (EMG) electrodes were implanted under deep anaesthesia (Pentobarbital, 60 mg/kg). Silver EEG electrodes were implanted through the skull on the dura above the parietal cortex and the cerebellum. Stainless steel EMG electrodes were placed under the skin on top of the neck muscles. Two stainless steel screws on the dura above the frontal cortex served as ground. A connector was fixed to three screws in the skull with dental cement. In a subset of six animals a calibrated temperature sensor was placed on the dura above the frontal cortex. In early November the animals were placed in continuous dim light (<1 lx) at 5.5°C (± 1). All animals entered hibernation, showing regular torpor bouts by early December.

EEGs were recorded in the animal's home cage. The cage was brought into the recording climate room at 5.5°C, after a period of 4 days of torpor at T_a of -5 ($n = 8$), 0 ($n = 4$), 5.5 ($n = 16$), 10 ($n = 11$) or 15°C ($n = 7$). After transfer, arousal was induced by gentle handling. The animal was removed from its cage and rectal body temperature (T_b) and cortical temperature (T_{crit}) was measured. The animal was subsequently handled until it showed regular voluntary movements. The electrodes were connected by flatcable via a slip ring swivel to an EEG/EMG amplifier system (EEG, 200 μ V/V, 0.2–80 Hz; EMG, 500 μ V/V, 20–600 Hz). EEG data were additionally filtered by a software low pass filter (-3 dB at 17 Hz, -35 dB/octave), sampled at 100 Hz, analyzed per 10 s epoch by fast Fourier transformation and later subjected to visual scoring of wakefulness and NREM and rapid-eye-movement (REM) sleep. Average NREM EEG power was calculated per 1 Hz bin over 0.2–15 Hz to assess spectral changes, and over the slow wave range (1.2–4 Hz). Power values were expressed relative to the power of the specific EEG frequency range during the individual's recording at 5.5°C. This resulted in EEG frequency activ-

ity values, either per frequency bin, or for the slow wave range alone (slow wave activity; SWA).

Animals were checked for spontaneous arousal episode occurrence by daily visual inspection. When an animal was warm or its position in the nest had changed, it was scored to have (had) an arousal on that day; when the animal was found torpid the next day, that day was scored as the first day in torpor. Since previous work indicated that after 4 days of torpor sleep debt was not maximal [9], we chose this torpor duration as standard situation. All EEGs were recorded at 5.5°C (± 1). For statistical analysis we used Kruskal–Wallis non-parametric ANOVA (KWNPA) for variation between groups, Mann–Whitney rank sum test (MWU) or Wilcoxon signed rank (WSR) test for group comparisons, and Spearman rank correlation (SRC).

An arousal episode consists of a rewarming phase and a euthermic phase. Since euthermic sleep may be dissimilar from torpor [4,5,9], we excluded EEG samples from the rewarming and recooling state. We used the end of the initial shivering EMG as border of the euthermic phase [9]. We defined the end of the euthermic phase as the time of the last substantial waking bout. Analysis of EEGs was restricted to the euthermic phase within these boundaries.

Results on spontaneous torpor bout duration (TBD), T_b and T_{crit} , euthermic phase duration (EPD) and NREM% and REM% over the whole euthermic phase are presented in Table 1. Spontaneous TBD was significantly reduced at T_a of both above and below 0–5.5°C (MWU, $P < 0.05$). Torpor T_b was higher at higher T_a . The difference between T_b and T_a was stable at $\sim 2^\circ\text{C}$ at T_a of 0°C and higher. The lowest T_b measured at -5°C was -0.6°C, showing that sustaining sub-zero T_b is not restricted to Arctic hibernators [1]. The T_b to T_a gradient was increased at -5°C compared to all other T_a (MWU, $P < 0.01$). Due to technical difficulties, T_{crit} was only measured in 3–6 animals in three T_a categories. The difference between T_{crit} and T_b was larger at -5°C (3.3°C, SEM 0.6; MWU, $P < 0.05$) than at 5°C (0.9°C, SEM 0.4) and 10°C (0.6°C, SEM 0.7). This indicates that animals specifically defend brain temperature: in Arctic ground squirrels both thorax and head region had relatively high temperatures [1]. The average

Table 1

Effects of T_a during torpor on spontaneous TBD (days), T_b and T_{crit} in torpor, EPD (h), NREM sleep % (NR%) and REM sleep % (R%) during induced arousal episodes after 4 days of torpor at that T_a

T_a (°C)	-5	0	5.5	10	15	<i>P</i> -value
TBD (days)	5.0 (1.5)	9.3 (1.8)	10.7 (0.7)	8.7 (0.6)	5.0 (0.0)	0.0007
T_b (°C)	0.3 (0.2)	2.0 (0.4)	7.5 (0.3)	11.9 (0.2)	16.9 (0.2)	0.0006
T_{crit} (°C)	3.5 (0.6)	–	8.0 (0.6)	12.3 (0.7)	–	0.022
EPD (h)	9.1 (0.5)	12.8 (2.0)	10.6 (0.6)	10.7 (1.5)	12.4 (1.4)	0.37
NR%	68.5 (2.5)	66.9 (3.4)	62.5 (1.1)	68.0 (1.8)	64.3 (1.9)	0.11
R%	10.5 (0.6)	10.8 (1.1)	10.0 (0.6)	9.3 (0.8)	8.4 (0.6)	0.27

Mean values (SEM) are given. The *P*-value indicates the test result of KWNPA on T_a groups.

Table 2

Average NREM (NR%) and REM sleep % (R%) for the first four euthermic 2 h intervals of induced arousal episodes after 4 days of torpor at T_a of $-5, 0, 5, 10$ and 15°C

T_a	-5		0		5.5		10		15		SRC +/-	
	NR%	R%	NR%	R%	NR%	R%	NR%	R%	NR%	R%	NR%	R%
0–2 h	87.9	2.6	87.3	4.1	70.9	2.6	77.9	2.6	56.8	1.3	–	ns
2–4 h	66.0	10.9	63.6	11.5	62.6	10.8	62.0	9.9	67.2	6.6	ns	ns
4–6 h	60.1	15.4	56.5	12.1	55.2	13.5	62.7	13.9	65.2	12.0	ns	ns
6–8 h	56.6	14.1	60.2	15.6	60.6	14.2	67.3	16.1	68.3	14.2	+	ns
SRC +/-	–	+	–	+	–	+	–	+	ns	+		

When KWNPA revealed significant variation, SRC was used to establish the direction of change. SRC results are shown as significant ($P < 0.05$) positive (+) or negative (–) correlations. ns indicates absence of significance.

T_{crit} over the euthermic phase was 31.5°C (SEM 0.5). The lowest euthermic T_{crit} after the first waking bout in euthermia was 29.6°C (SEM 0.7), close to the T_{crit} at the end of the EMG surge (28.3°C , SEM 0.9; WSR, $P = 0.09$). The time lag between the end of the initial EMG surge to the lowest euthermic level was 10.1 min (SEM 4.5). Note that the T_{crit} was assessed by a thermistor on the dura (not inserted into the cortex), and exposed to low T_a . The values may not reflect T_{crit} levels in intact animals. NREM% and REM% over complete euthermic phases were not distinguishable between T_a categories. Data on the pattern of NREM% and REM% over the first four euthermic 2 h intervals are presented in Table 2. REM% consistently increased at all T_a . No differences in REM% were found within any 2 h interval between T_a categories. NREM% showed a decrease with progress of time in euthermia, except in the 15°C T_a category. Within the first 2 h interval a negative and in the fourth 2 h interval a positive correlation was found between NREM% and T_a . In the first 2 h interval, animals showed more NREM sleep after torpor at T_a below 15°C .

The NREM EEG power spectra for the first three euthermic 2 h intervals are shown in Fig. 1. In the fourth 2 h interval no significant differences changes occurred between T_a categories. Variation (KWNPA, $P < 0.05$) with a negative direction in time was observed within the 1.2–6 Hz range for the $-5, 0, 5$ and 10°C T_a categories, and not in the 15°C group. Both the $-5, 0, 5$ and 10°C T_a categories and the 15°C category showed significant variation in the 7.2–15 Hz range. NREM EEG frequencies after torpor at $-5, 0, 5.5$ and 10°C showed negative correlations with subsequent euthermic 2 h intervals for 0.2–6 Hz, and positive correlations for 7.2–15 Hz (SRC, $r^2 > 0.17$, $n = 144$, $P < 0.05$). The 15°C spectra showed only positive correlations from 3.2–15 Hz (SRC, $r^2 > 0.39$, $n = 26$, $P < 0.05$). Thus, the pattern of difference in the course of euthermic time is an initial reduction of frequencies above 7 Hz after torpor at all T_a , and an increase in frequencies below 7 Hz after torpor at T_a of -5 – 10°C , which does not happen after torpor at 15°C . Analysis of spectral differences between T_a categories showed that the 1.2–2 Hz bin was higher in the first two euthermic

2 h intervals after torpor at -5°C (MWU, $P < 0.05$). NREM EEG after torpor at 15°C was characterized by lower activity of all frequencies during the first euthermic 2 h interval, and a continued reduction in the slow wave range.

SWA in the first and the last euthermic 2 h interval are shown in Fig. 2. Initial SWA, e.g. SWA during the first euthermic 2 h interval, was significantly higher after torpor at $-5, 0, 5.5$ and 10°C , compared to the final SWA in the last 2 h interval just prior to reentry to torpor (MWU, $P < 0.05$). After torpor at 15°C no difference between initial SWA and final SWA was found. Initial SWA after torpor at -5°C was higher compared the initial SWA of the higher T_a categories (MWU, $P < 0.05$). Initial SWA after torpor at 15°C was lower compared to initial SWA following torpor at lower T_a (MWU, $P < 0.05$).

T_a affected spontaneous TBD, and some of the sleep variables during arousal episodes. Spontaneous TBD was reduced at sub-zero T_a . Specifically in this condition several other variables changed, in torpor and in the euthermic phase. The T_b to T_a gradient was increased in torpor, as also reported in the Cascade ground squirrel [6]. The T_{crit} and the internal gradient between T_b and T_{crit} were enhanced.

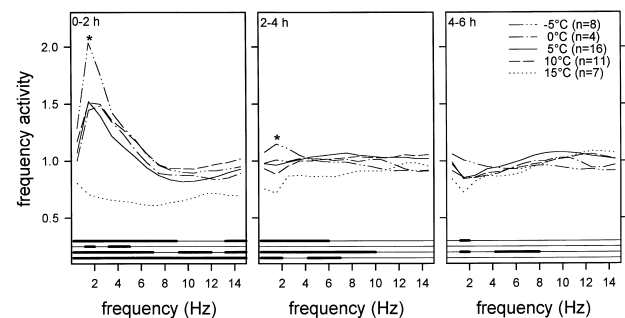


Fig. 1. Frequency activity per 1 Hz bin over 0.2–15 Hz during the first three euthermic 2 h intervals of arousal episodes, induced after 4 days of torpor at T_a ranging from -5 to 15°C . *Significance of the difference of the -5°C T_a category from the other categories (MWU, $P < 0.05$). The heavy lines at the bottom of the three panels indicate the frequencies where significant differences were observed between the 15°C ($n = 7$) category and -5°C (upper line, $n = 8$), 0°C ($n = 4$), 5.5°C ($n = 16$) and 10°C ($n = 11$) categories, respectively (MWU, $P < 0.05$)

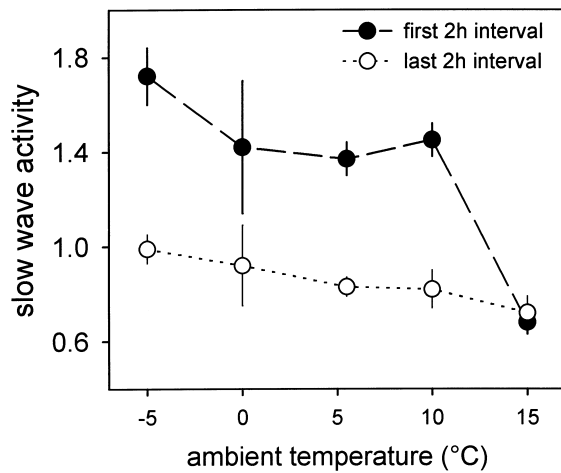


Fig. 2. SWA during the initial and final euthermic 2 h interval of arousal episodes as a function of the T_a during the 4 days of preceding torpor. The final 2 h interval was the last euthermic 2 h interval prior to reentry into torpor.

Moreover, an increase of SWA in the first two euthermic 2 h intervals of arousal episodes occurred, as compared to all other T_a categories (see Fig. 1). This temperature dependence of SWA might be attributed to metabolic activity in the warming up phase [10]. Indeed, animals were observed to shiver over a longer period rewarming from torpor at -5°C compared to other T_a . Such a hypothesis can, however, not account for the absence of an increase in SWA after torpor at 15°C , where substantial shivering also occurred.

Spontaneous TBD was shorter both at the lower (-5°C) and the upper end (15°C) of our temperature range. If spontaneous TBD would be determined by the rate of sleep debt increase in torpor we would have expected higher initial SWA both after torpor at -5 and 15°C . This was observed only at -5°C . If anything, initial SWA declined as a function of T_a during the preceding torpor. After torpor at 15°C no elevation of initial SWA whatsoever could be observed.

Larkin and Heller [8] recorded EEGs in Golden-mantled ground squirrels during spontaneous arousal episodes at T_a of 11 – 21°C . Their correlative analysis reveals close similarity to our experimental data: after torpor at 21°C SWA did not increase, while it did at 11°C . The authors concluded that some factor other than sleep homeostasis regulates arousal episode occurrence. Both data sets appear consistent with the idea that the SWA enhancing effects

are restricted to temperatures below $\sim 15^\circ\text{C}$. It is tempting to speculate that the absence of hypothalamic neuronal activity below 14 – 18°C [7] is related to this fact.

In conclusion, the temperature dependent variations in arousal frequency can not be attributed to a temperature dependence of sleep debt increase. This makes sleep debt unlikely to be involved in the regulation of arousal episode occurrence. It does however not imply that sleep during arousal episodes has no relevance for recuperation from effects induced by torpor.

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