

## Warming up for sleep? — ground squirrels sleep during arousals from hibernation

Serge Daan<sup>1</sup>, Brian M. Barnes<sup>2</sup> and Arjen M. Strijkstra<sup>1</sup>

<sup>1</sup>Zoologisch Laboratorium, Universiteit Groningen, Haren (The Netherlands), <sup>2</sup>Institute of Arctic Biology, University of Alaska, Fairbanks, AL 99775 (U.S.A.)

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Hypothermia during mammalian hibernation is periodically interrupted by arousals to euthermia, the function of which is unknown. We report that arctic ground squirrels (*Spermophilus parryii*) consistently sleep during these arousals, and that their EEG shows the decrease in slow wave activity ( $\delta$  power) that is characteristic of a declining requirement for sleep. These results are consistent with the novel hypothesis that the need for sleep slowly accumulates during torpor, and that returning to euthermia is periodically required to allow sleep. Sleep thus seems to be energetically expensive for a hibernating mammal, and cannot be considered solely a strategy for saving energy.

Hypothermia during mammalian hibernation is not continuous; instead, throughout the hibernation season animals periodically increase body temperature from near 0°C during torpor to 36–38°C during so-called 'arousals' (Fig. 1). Although euthermia during these arousals usually only lasts < 20 h [1, 12], the energy expended in warming up and maintaining high body temperatures during arousals is estimated to represent up to 90% of the total energy spent during the entire hibernation season [6, 15, 21]. This expense, and the fact that all hibernators yet investigated show such spontaneous arousals [18], suggest that they serve a critical function. Yet, despite considerable theoretical and experimental effort, their functional significance remains unknown [23]. Propositions that arousals are required to eliminate metabolic waste products [9, 10], to replenish blood glucose levels [13], or to restore cellular electrolyte balance [11] have not survived critical experimental testing [23]. We propose, in a new theory, that the normal restorative function of sleep is inhibited at the low brain temperatures of torpor, but the need for sleep continues to accumulate, albeit at a reduced rate. Periodic returns to euthermia are then necessary to allow the required sleep.

Evaluation of these hypotheses will eventually involve experimental approaches that manipulate sleep deficit, arousal sleep, torpor duration, and ambient and brain

temperatures in hibernators. In this initial report we concentrate on two predictions from the hypotheses. The first is that hibernators should devote the majority of time in euthermic arousal to sleep. Heretofore, no electroencephalogram (EEG) recordings have been made during complete spontaneous arousals from hibernation that would allow evaluation of this prediction. Mammals enter hibernation while predominantly in slow-wave, or non-rapid eye movement (non-REM) sleep, at least until brain temperatures decrease below 25°C, when EEG amplitude diminishes and the assignment of vigilance state becomes impossible [20]. During deep torpor the EEG is silent, although single cell recordings reveal sleep-like bursting within the thalamus [16]. These and other observations [17] have led to the assumption that hibernation is an extension of sleep and that periodic arousals represent waking [3, 14]. Since the issue should be resolvable with conventional polygraphic criteria, we carried out EEG and EMG measurements during arousals from hibernation in the Arctic ground squirrel *Spermophilus parryii*.

The second prediction tested is that the intensity of sleep should decrease during the arousal, due to the decline of the sleep deficit [7]. While there is no known measure of intensity for REM-sleep, non-REM sleep intensity is reflected in the EEG power density in the delta ( $\delta$ ; 1–4 Hz) range [4]. The typical decline in this power density during sleep episodes is interpreted as the gradual completion of restorative processes [4, 7]. If sleep debt continuously accumulates during torpor inter-

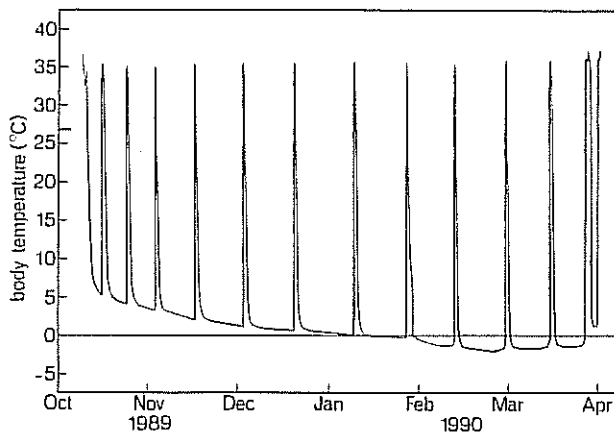


Fig. 1. Body temperature of an arctic ground squirrel, hibernating in a self-dug burrow in an outdoor enclosure in Fairbanks, Alaska, U.S.A., indicated by an abdominal temperature-sensitive radiotransmitter [2]. Low body temperature during torpor is interrupted each 1–3 weeks by brief (8–16 h) elevations to euthermic levels (periodic arousals).

vals, then non-REM intensity should decline during arousal sleep. In addition to testing these predictions, we compared sleep and non-REM intensity during arousals following short and long intervals of torpor.

Arctic ground squirrels were collected in late summer on the North slope of the Brooks range in Alaska, and brought to the laboratory in Haren. They were maintained individually in cages with nesting material in a temperature controlled cabinet at 2°C (S.D. 1°C) in continuous darkness. In November, after they had begun to hibernate, animals were implanted with chronic EEG and EMG electrodes. EMG electrodes shielded in silastic tubing (Plastic Products Co.) were placed subcutaneously over the neck muscles, and silver EEG electrodes were implanted on the dura above the parietal cortex and above the cerebellum while animals were under pentobarbital anesthesia (60 mg/kg, i.p.). Two symmetrically placed stainless-steel screws above the

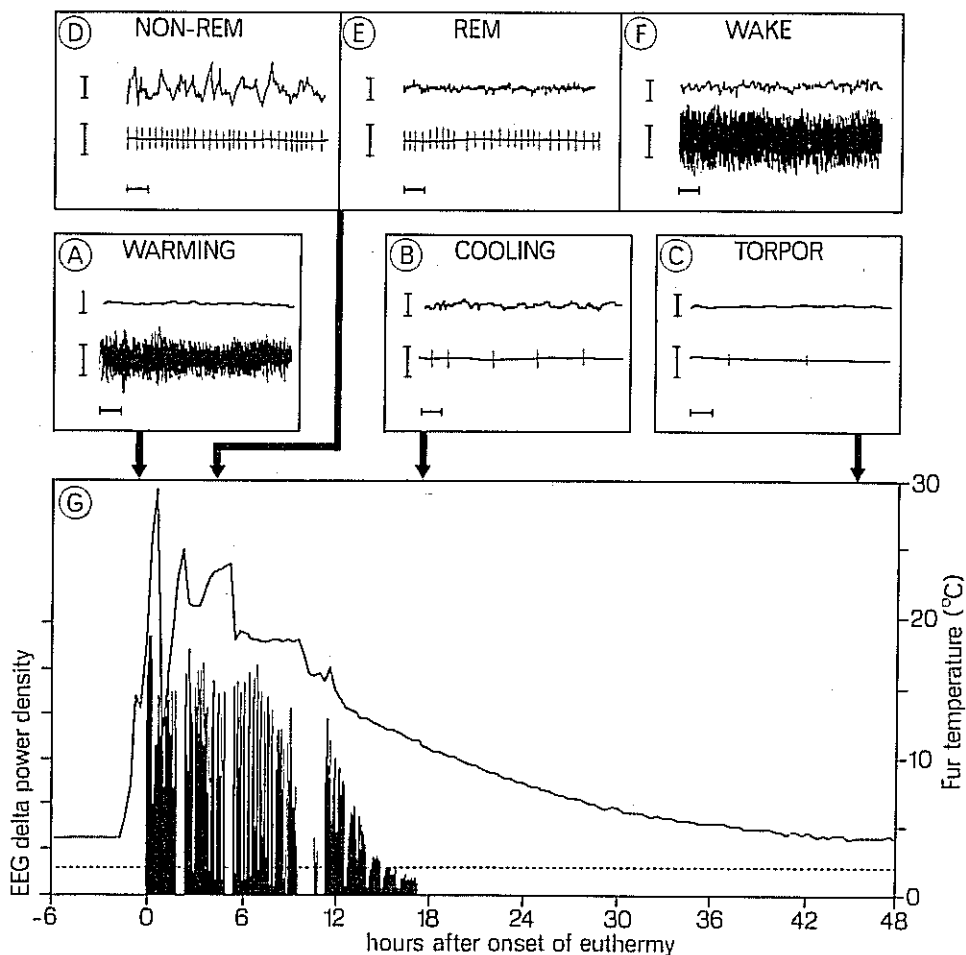


Fig. 2. Upper panels: examples of EEG and EMG records in three stages of a periodic arousal by a hibernating arctic ground squirrel. Each box shows a 10 s interval of simultaneous EEG (upper trace) and EMG (lower trace) recordings: during rewarming from torpor (A); during reentry into torpor (B); during torpor (C); during non-REM sleep (D), REM sleep (E) and wakefulness (F) in euthermia. Spikes in EMG traces in the non-REM/REM sleep and Cooling/Torpid panels indicate heart beats. G: fur temperature and SWA during a typical periodic arousal; time 0 defined as fur temperature halfway between torpid and euthermic values.

frontal cortex served as a ground. Surgery was done one day after removing animals from the cold, and recovery at room temperature was allowed for 7–10 days before animals were returned to the cold. From December 15, 1989, until April 1, 1990, ground squirrels were monitored for the occurrence and duration of spontaneous arousals, or induced to arouse by handling after 5–7 days of torpor. The occurrence of torpor and arousals was monitored by a thermistor which was loosely placed in the ventral fur. We defined the onset of euthermia in arousal as the time when fur temperature was half-way between the torpid levels and maximal levels reached in euthermia. EEG and EMG were recorded on an Elema Schonander polygraph with a paper speed of 10 mm/s and filter settings 30 Hz, time constant 0.6 s for the EEG, and 700 Hz, time constant 0.015 s for the EMG. Beginning during rewarming to euthermia, which lasted 4–6 h, EEG and EMG were recorded continuously on a polygraph for 24 h, which was usually sufficient to let the animal reenter torpor again. Power densities of the EEG were recorded in nine frequency bands covering the range from 0.75 to 13.5 Hz [8].

Fig. 2 presents examples of EMG and EEG paper chart records during three stages of an arousal: warming up, euthermia, and cooling. The warming up stage was characterized by virtually zero amplitude EEG and high EMG levels associated with shivering thermogenesis (Fig. 2A). Cooling was associated with a decreasing amplitude of the EEG, while the flat EMG suggests muscle atonia (Fig. 2B,C). During euthermia, i.e., the stage of high body temperature in the middle of the arousal, three vigilance states, of REM sleep, non-REM sleep, and wakefulness (WAKE), were identified: Non-REM is characterized by low EMG and high EEG amplitude (Fig. 2D), REM by very low EMG and low EEG amplitude (Fig. 2E) and WAKE by high EMG and low EEG amplitude (Fig. 2F). Euthermia in arousals lasted on average 18.2 h (S.D. 7.7;  $n=7$ ), and was largely dedicated to sleep. The first interpretable EEG occurred approximately 2 h into the arousal at 15–20°C fur temperature and was of non-REM. The first  $\sim 2/3$  of the euthermic interval was mainly spent cycling between non-REM and REM, with a mean cycle length of 0.23 h (S.D. 0.03;  $n=7$  arousals in 3 animals). WAKE occurred primarily during the last 1/3 of the arousal. The proportion of time spent in the different vigilance states during euthermia was 53.0% (S.D. 3.8%) for non-REM, 14.1% (S.D. 4.3%) for REM and 32.9% (S.D. 7.4%) for WAKE. Behaviorally, the animals spent most of the arousal in a curled hibernation posture, as has been documented previously in *S. lateralis* [19].

Slow wave activity (SWA) during non-REM, defined as the EEG power density in the range 1.5–3.5 Hz, de-

clined gradually in euthermia, and then more rapidly as cooling began (Fig. 2G). This decline was characteristic of all 6 arousals for which we analyzed SWA. For the combined data the hourly average of SWA during non-REM, and before substantial wakefulness, was expressed as the log of the fraction of the mean value over 12 h (Fig. 3). The first substantial wake interruption lasting at least 0.5 h occurred between 5 h and 14 h into euthermia, and caused the declining number of values indicated above the data points. There was a significant decline in SWA over time ( $T$ , h) within arousals ( $\log \text{SWA} = 0.050 - 0.011T$ ;  $n=52$  hourly SWA values;  $P < 0.005$ ; two-tailed). This pattern of decline in SWA during sleep is similar to that seen during the night in arctic ground squirrels during their non-hibernation season, and to that seen in other rodents during the rest phase of their circadian cycle [8].

The devotion of most of periodic arousals to sleep and the decline of SWA during that sleep in the present experiments are consistent with the hypothesis that hibernators periodically arouse from hibernation in order to repay a sleep debt. Because torpid animals are presumed to be continuously sleep deprived, further support would include experimental evidence that SWA and sleep time during euthermia are less after short compared to long intervals of torpor. In a pilot study, 4 induced arousals that followed an average of 5.9 days of torpor, showed a sleep time of 62% (S.D. 8.6) during the first 6 h, as compared with 74% (S.D. 5.6) in 4 spontaneous arousals that followed 12.7 days of torpor. SWA in non-REM sleep was on average 7% less (S.D. 7.5%;  $n=3$ ) in these induced as compared with spontaneous arousals.

These data might also be consistent with other hypotheses for the function of periodic arousals. There may be other restorative processes taking place during arousal that require high body temperature but not

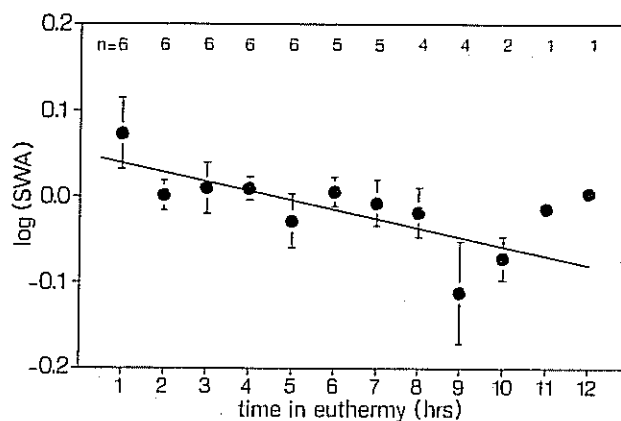


Fig. 3. Time course of slow-wave activity (SWA) during the first 12 h of 6 arousals in 3 hibernating arctic ground squirrels. Means ( $\pm$  S.E.M.) of the values for different arousals are shown.

sleep, and with which sleep co-occurs passively, as an epiphenomenon. Such non-specific hypotheses, however, do not explain why non-REM sleep intensity should be highest at the beginning of the arousal, and appears to be greater following long versus short torpid intervals. Whatever the alternative process would be, it does not require wakefulness, and it is performed at the body temperature typical of nocturnal sleep (ca. 35°C) rather than that of diurnal wakefulness (ca. 37°C) in summer (Fig. 1).

If the need for sleep does build up during torpor, it apparently does so much more slowly than during euthermic wakefulness. This suggests that increases of the sleep regulating variable [7] may be related to either metabolic rate and/or brain temperature. Manipulations of metabolic rate and brain temperature during torpor and their influence on sleep patterns during arousals should be revealing for these relationships. The proposition that periodic arousals from hibernation occur to allow for sleep at high body temperatures provides a novel functional explanation for the arousal phenomenon, and, perhaps, new insight into the function of sleep. It suggests that the term 'periodic arousal', although generally accepted, may be a misleading description, and that 'periodic euthermia' is a less connotative expression. Sleep has been proposed to have a primary function in saving energy [3, 14] or to be a memory regulating process [e.g., 5]. That hibernating mammals would temporarily abandon the maximal energy savings strategy of quiet dormancy to achieve sleep cannot easily be reconciled with these hypotheses in their pure, exclusive, form, but makes good sense in the context of a restorative function of sleep [7, 22].

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- 1 Barnes, B.M., Kretzmann, M., Licht, P. and Zucker, I., The influence of hibernation on testis growth and spermatogenesis in the Golden-mantled ground squirrel, *Spermophilus lateralis*, *Biol. Reprod.*, 35 (1986) 1289-1297.
- 2 Barnes, B.M., Freeze avoidance in a mammal: Body temperatures below 0°C in an Arctic hibernator, *Science*, 244 (1989) 1593-1595.
- 3 Berger, R.J., Comparative aspects of energy metabolism, body temperature and sleep, *Acta Physiol. Scand.*, 133 Suppl. 574 (1988) 21-27.

- 4 Borbély, A.A., A two-process model of sleep regulation, *Human Neurobiol.*, 1 (1982) 195-204.
- 5 Crick, F. and Mitchison, G., The function of dream sleep, *Nature*, 304 (1983) 111-114.
- 6 Daan, S., Activity during natural hibernation in three species of Vespertilionid bats, *Neth. J. Zool.*, 23 (1973) 1-71.
- 7 Daan, S., Beersma, D.G.M. and Borbély, A.A., Timing of human sleep: recovery process gated by a circadian pacemaker, *Am. J. Physiol.*, 246 (1984) R161-R178.
- 8 Dijk, D.J. and Daan, S., Sleep EEG spectral analysis in a diurnal rodent: *Eutamias sibiricus*, *J. Comp. Physiol. A*, 165 (1989) 202-215.
- 9 Dubois, R., *Physiologie comparée de la Marmotte*, Masson, Paris, 1896.
- 10 Fisher, K.C., On the mechanism of periodic arousal in the hibernating ground squirrel, *Ann. Acad. Sci. Fenn. Ser. A*, IV, 71 (1964) 141-156.
- 11 Fisher, K.C. and Manery, J.F., Water and electrolyte metabolism in heterotherms. In K.C. Fisher, A.R. Dawe, C.P. Lyman, E. Schonbaum and F.E. South (Eds.), *Mammalian Hibernation III*, Elsevier, New York, 1964, pp. 235-279.
- 12 French, A.R., Allometries of the durations of torpid and euthermic intervals during mammalian hibernation: a test of the theory of metabolic control of the timing of changes in body temperature, *J. Comp. Physiol. B*, 156 (1985) 13-19.
- 13 Galster, W.A. and Morrison, P., Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel, *Am. J. Physiol.*, 218 (1970) 1228-1232.
- 14 Heller, H.C., Sleep and hypometabolism, *Can. J. Zool.*, 66 (1988) 61-69.
- 15 Kayser, C., L'hibernation des mammifères, *L'Année Biol.*, 29 (1963) 109-150.
- 16 Krilowicz, B.L., Glotzbach, S.F. and Heller, H.C., Neuronal activity during sleep and complete bouts of hibernation, *Am. J. Physiol.*, 255 (1988) R1008-R1019.
- 17 Krueger, J.M. and Shoham, S., Homologies between sleep factors and hibernation triggers, in H.C. Heller, X.J. Musacchia and L.C.H. Wang (Eds.), *Living in the Cold: Physiological and Biochemical Adaptations*, Elsevier, New York, 1986, pp. 235-244.
- 18 Lyman, C.P., Willis, J.S., Malan, A. and Wang, L.C.H., *Hibernation and Torpor in Mammals and Birds*, Academic Press, New York, 1982.
- 19 Torke, K.G. and Twente, J.W., Behaviour of *Spermophilus lateralis* between periods of hibernation, *J. Mammal.*, 58 (1977) 385-390.
- 20 Walker, J.M., Glotzbach, S.F., Berger, R.J. and Heller, H.C., Sleep and hibernation in ground squirrels (*Citellus* spp): electrophysiological observations, *Am. J. Physiol.*, 233 (1977) R213-R221.
- 21 Wang, L.C.H., Time patterns and metabolic rates of natural torpor in the Richardson's ground squirrel, *Can. J. Zool.*, 57 (1978) 149-155.
- 22 Webb, W.B., Theories of sleep functions and some clinical implications. In R. Drucker-Colin, M. Shkurovich and M.B. Serman (Eds.), *The Functions of Sleep*, Academic Press, New York, 1979, pp. 19-35.
- 23 Willis, J.S., The mystery of the periodic arousal. In C.P. Lyman, J.S. Willis, A. Malan and L.C.H. Wang (Eds.), *Hibernation and Torpor in Mammals and Birds*, Academic Press, New York, 1982, pp. 92-101.