The Role of the Suprachiasmatic Pacemaker (SCN) in Energy Expenditure During Hibernation of Golden-mantled Ground Squirrels

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Abstract. Torpor bouts and brief euthermic arousal episodes alternate in a precise sequence during hibernation of golden-mantled ground squirrels. The characteristics of arousal episodes were quantitatively analyzed for squirrels in natural habitat and also for a group maintained in a simulated natural environment in the laboratory. Wild golden-mantled squirrels, which were completely SCN-lesioned and repatriated in autumn, were not recovered in the field population in spring. The SCN pacemaker apparently fine-tunes the temporal pattern of core body temperature during hibernation and may thereby help in rationing out the vital body fat supply through winter.

Introduction

The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and has an important temporal regulatory function in many behavioral and physiological processes (reviews in Weaver, 1998; Takahashi et al., 2001; DeCoursey, 2003). In at least three species of squirrels, the SCN is important for their survival in natural habitats (DeCoursey, et al., 2000). SCN involvement in timing of mammalian hibernation has also been shown at least under laboratory conditions (Ruby et al., 1998).

Many small rodents living in cold climates escape the rigors of winter by fattening before entering a state of long-lasting deep torpor in a deep burrow below the freeze line (Barnes, 1989; Strijkstra, 1999; Ruby, 2003). Through the use of a greatly reduced metabolic rate during the time when food and water are unavailable, the animals can survive autumn drought and winter periods (Körtner and Geiser, 2000). Although the hibernation season may last up to nine months, torpor is interrupted by brief, recurring arousal episodes that usually persist for less than 24 h Barnes, 1989; Michener, 1994). The fact that the metabolic cost of arousal episodes is estimated at 86% of total energy expenditure in European ground squirrels (Strijkstra, 1999) implies that precise arousal episode timing is essential in hibernation.

The golden-mantled ground squirrel of the Pacific Cascade Mountains provides an excellent model for examining the function of the SCN pacemaker in energy expenditure during hibernation. The squirrels are highly adapted in their annual cycle to their favored habitat in the 'High Desert' Ponderosa Pine forests. Much is known about SCN regulation of daily activity patterns and about some of the behavioral and physiological events of hibernation in lab-maintained golden-mantled squirrels (Ruby et al., 1998), but little is known for free-living individuals.

The hibernation bout pattern data presented here are based on a study of a population of golden-mantled squirrels from wilderness habitat and from a parallel group maintained in a laboratory Hibernaculum under simulated natural conditions. Intact and surgical controls were compared to SCN-lesioned animals.

Material and Methods

In the field study, squirrels were studied for four seasons at a pristine, 20-hectare forest site in the Metolius Research Natural Area, near Camp Sherman, OR. In this ideal habitat, population levels of golden-mantled squirrels are high and very stable. Permanent trapping cairns sheltering National live traps were set out in a grid at 50-m intervals in order to gather data from all resident animals at regular intervals during the snow-free season from May to October.

Approximately 80 animals inhabited the area in early spring; about 250 were present after emergence of young in early summer. All animals captured on the study site were marked for individual recognition.

A walk-in cold room was used for the Hibernaculum to house squirrels in individual cages. The chamber was programmed to track deep ground ambient temperature of the Metolius RNA site on a daily basis. A light schedule was provided, based on daily sunrise/sunset times for Bend, OR, with DD from October 15- to March 1.

All Project Animals selected for intensive study of hibernation patterns were equipped from September 15 through May 15 with a telemetric data logger, implanted intraperitoneally (Thermochron iButton from Dallas-semiconductor), which monitored body temperature continuously at 3-h intervals. Extensive efforts were made in the field studies to maximize the retrieval of the data loggers. The protocol consisted of three groups: Intact Controls, Surgical Controls (Sham SCN-X) and the SCN-lesioned experimental group. Golden-mantled squirrels for Hibernculum studies were trapped at a site 3 km from the field study site. All animals held in captivity were housed in shoebox style cages with sufficient food and water to maintain body weight at the level of field animals.

For SCN surgery, animals under deep anesthesia were mounted in a Kopf stereotaxic instrument for alignment of the electrode. The posterior edges of the two orbital zygomatic processes served as a precise skull landmark. Lesion coordinates were determined by constructing a histological atlas for the ventral hypothalamic region by serial histological sectioning of SCN-lesioned calibration animals. A 3-mm diameter skull hole was drilled for insertion of the lesion electrode. Platinum-iridium electrodes from FHC were employed with a current of 25 mv applied for 20 sec.

Results

Field data is based on a total of 30 iButtons recovered from 28 individuals after termination of winter hibernation, plus partial life history data from 54 non-recovered field animals. The field numbers for retrieved Project Animals include 18 intact controls, five surgical controls and five squirrels with partial SCN lesions. The overall recovery rate was 35.7% based on pooled data for all years. The 31 animals used in the Hibernaculum included 12 intact controls, two surgical controls, and 17 SCN-lesioned individuals.

Precise, predictable patterns of hibernation bouts and arousal episodes occurred in all field intact control animals. Almost all individuals dropped abruptly from the autumn euthermic level into hibernation, although a few exhibited one or more shallow test drops. Hibernation then proceeded with a regular succession of torpor bouts and euthermic arousal episodes at intervals from 1-15 days. Two distinct sequence patterns are evident. Adult females progressed through a series of hibernation bouts, with body temperature tracking about 2^{o}C above the deep ground temperature. The modulation of the bouts was directly related to ambient deep ground temperature: the colder the ambient temperature, the longer the hibernation bouts (Fig. 1A). The pattern for juveniles of both sexes was similar to adult females (Fig. 1B). A contrasting picture was seen in adult males. Although adult males disappear from the surface in fall droughts, they often do not enter torpor until late November. Adult males rarely decreased their body temperature below 10^{o}C, which was well above the ambient ground temperature. They usually aroused from hibernation in mid-February when ambient deep ground temperatures were still dropping (Fig. 1C). Their torpor bouts were also quite short.

(**Insert Figure 1 about here).**

A consistent pattern for all field controls is clear for the total duration of the hibernation season, the number of arousal bouts, and the average duration of hibernation bouts (Fig. 2). Females and juveniles hibernated for about 7 months, with 20–25 torpor bouts averaging 8-10 days and arousal episodes lasting about 24 h. For breeding males, hibernation generally lasted 3 months, the number of bouts was less, and the mean duration was shorter. Modulation of the frequency of hibernation bout length in males was also much less evident. The number of aberrations in pattern of arousal episodes was low for all controls.

Only five SCN-lesioned field squirrels were ever recovered in the 4 years of the study and all were only partially lesioned: three females ranging from 10%-50 % SCN remaining, and two males with 20% and 25% SCN remaining. For this reason, the data for SCN effects must come from the Hibernaculum data.

(Insert Figure 2 about here.)

Hibernaculum control animals closely resembled field animals in their hibernation duration time, number of bouts, and length of bouts relative to minimal ambient temperature. A major difference between control and SCN-X animals was the presence of aberrant euthermic periods during hibernation (Fig. 3). These abnormal patterns were less pronounced for animals that had undergone SCN-X surgery in early fall than for those lesioned earlier in the year or held more than one year in the Hibernaculum. They ranged from very irregular torpor bouts, to extended euthermic arousal episodes in midwinter, and even a total lack of hibernation.

(Insert Figure 3 about here.)

The relative wakefulness of SCN-X animals potentially heightened the risk of starvation since extra stored fat was required for the extended arousal episodes. After the unexpected death of an early waking intact control male in the Hibernaculum, regular feeding was initiated for each individual as soon as it terminated hibernation. As long as normal feeding was maintained immediately upon arousal from hibernation, the only difference in survival seen between Intact Controls and SCN-lesioned squirrels in the Hibernaculum was the inadvertent starvation of this one control male (Fig. 4). The difference of survival rate between completely SCN-lesioned animals in the field and in the Hibernaculum was one of the most surprising results of the study.

(Insert Figue 4 about here.

Discussion

Practically no information is currently available concerning the involvement of the SCN pacemaker in hibernation of any wild mammalian species living in natural habitat (DeCoursey 2000). The lack stems from the extreme difficulty of repatriating SCN-lesioned individuals at their home site after surgery and then assessing their behavioral and physiological performance for the remainder of their lifetime. Telemetric tracking methods such as radio tracking or data logging during the extended period of hibernation are possibilities, but these methods are extremely arduous, time-consuming and expensive (Michener, 1992; Strijkstra, 1999; DeCoursey et al., 2000). As a result, most data comes from studies of laboratory-reared animals under artificial, non-ecological conditions of temperature, light schedule, and feeding.

Laboratory studies have been helpful in characterizing some features of mammalian hibernation, but a comprehensive understanding needs a view of a wild, free-living animal against the backdrop of its natural environment. The holding of golden-mantled squirrels for several years in the laboratory may have conditions that differ greatly from natural field conditions: a constant, relatively high temperature such as 10ºC. (Grahn et al., 1994) or 6.5ºC, under LD or LL light regimes, with ad libitum food supplied (Ruby et al., 1998). For the current study, a pristine site field site free of interference from humans or their domesticated animals was considered an essential design component. Similarly, the Hibernaculum was programmed to simulate as closely as possible the annual temperature and lighting cycle of the actual hibernation burrows at the field site.

The two hibernation patterns of intact individuals reflect life history features, particularly energy expenditure and reproductive constraints. In the brief mountain summertime, the squirrels must breed and store enough body fat to last through frequent autumn droughts and bitter winter seasons. Breeding males are photoperiodic and require about eight weeks exposure to increasing day length in the preparatory phase of gonadal growth. Energy source during this extremely cold period is unknown. The iButton field records indicate resumed euthermy as early as mid-February for adult males but no sight records of squirrels above ground or evidence for a winter food cache in a male's burrow under field conditions are available. In the present study, some Hibernaculum squirrels of both genders stored a small pile of sunflower seeds but most refrained from eating during hibernation. In contrast, adult females arouse from hibernation and mate immediately upon coming to the surface in late April or early May. They seemingly benefit from prolonged hibernation, since a secure surface food supply of spring greens is then available to support them through the subsequent energetically draining gestation and lactation periods. The juveniles can likewise ill afford coming to the surface when a food supply is questionable.

The exact fate of the SCN field project animals is unknown. Extensive trapping failed to relocate any completely lesioned individuals during spring recovery trapping. In contrast**, 27.4%** of intact animals and surgical controls were retrieved, which is a typical result in most capture-recapture studies. The Hibernaculum results give a possible explanation. SCN-lesioning carried out shortly before the start of hibernation had relatively little effect on the body temperature record, and usually caused only slight extensions of the time of euthermia during an arousal episode. In the second year following lesioning, several severe aberrations in body temperature patterns occurred including continuous euthermia with a total lack of hibernation bouts (unpublished data in progress). As long as a small food reserve was supplied, all SCN-lesioned Hibernaculum individuals survived.

Data from body weights of Hibernaculum animals throughout hibernation suggest strongly that almost all squirrels are at risk of starvation at the time of arousal from hibernation. The metabolically costly arousal mechanism of even intact controls may bring an individual close to the lethal zone. Any aberrations in euthermy extension during hibernation may tip the balance particularly for the early arousing breeding males. Juveniles may enter hibernation with an insufficient fat supply after drought-racked summers and be quite vulnerable to starvation during hibernation. Certainly non-hibernating SCN-X individuals could not survive a winter under field conditions. This fact may help explain the failure to recover any fully lesioned individuals in the field.

In summary, modifications of hibernation pattern and accompanying body weight losses in SCN-deleted animals could result in death by starvation at the critical termination of hibernation in late winter, unless supplementary food is provided. The function of the SCN-pacemaker may be to fine tune hibernation bout pattern for greatest efficiency in energy expenditure.

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Figure legends

Figure 1. Representative hibernation pattern of body temperature: A. Adult female; B. Juvenile; and C. Adult male.

Figure 2. Parameters of hibernation: A. Total duration; B. Number of bouts; C. Duration of bouts. Symbols: black = adult females; white = juveniles, and hatched = adult males

Figure 3. Hibernation aberrations in SCN-lesioned squirrels. A. Multiple test drops and extended euthermia; B. Irregular arousal bouts; and C. Extended arousal bout.

Figure 4. Survival rate of Intact Controls and SCN-lesioned squirrels at the Field Project site (left) and in the laboratory Hibernaculum (right). Symbols: black = SCN-X, white = Intact Control.

Figure 1.

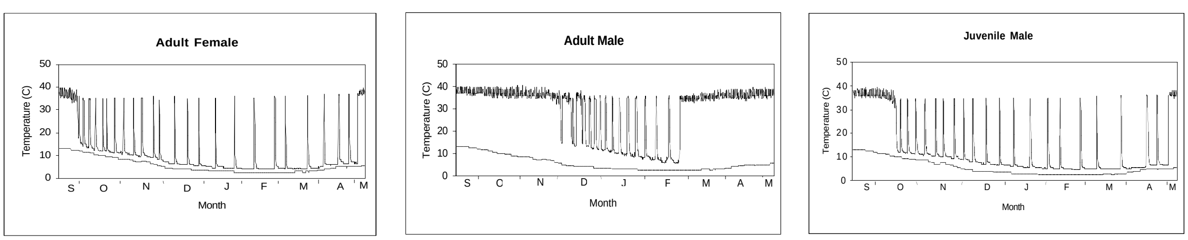

Figure 2.

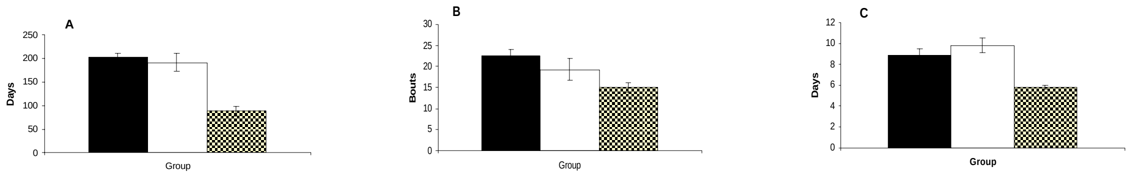

Figure 3.

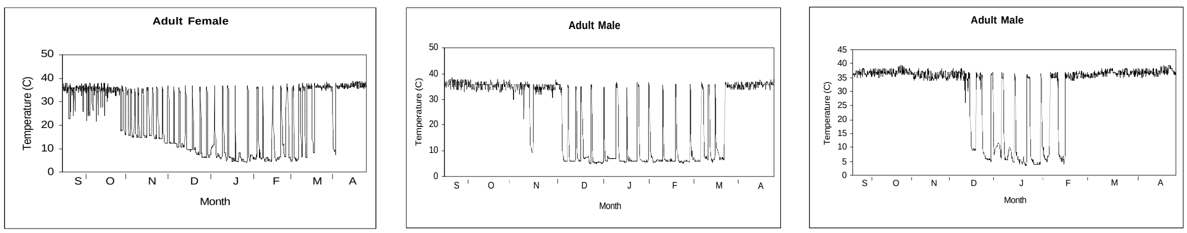

Figure 4.