Resetting the Clock

After hibernation, arctic ground squirrels may need the light of day to reactivate body rhythms.

By Cory T. Williams and C. Loren Buck

As the farthest-north hibernating small mammals in North America, arctic ground squirrels (*Urocitellus parryii*) are exposed to profound seasonal changes in light and ambient temperature. Their annual cycle includes a short summer season of three to five months. During that time individuals are active aboveground and maintain a favorably warm, or "euthermic," body temperature of between 96 and 104 degrees Fahrenheit. Animals spend the remainder of the year sequestered in frozen burrow systems, during which their body temperatures fluctuate, in what is known as "heterothermy." For long bouts (two to three weeks) they are in a continuous state of torpor, reducing their metabolic rate and maintaining a body temperature below that of an ice cube. Their torpor is interrupted by brief, ten- to twenty-hour intervals during which their body temperature is elevated to a euthermic level.

For two to three weeks before first emerging to the surface from their burrows, males terminate heterothermy (the lowering and raising of their body temperature), remain euthermic, and start feeding on food they have stored. During this time, they regain their body condition and undergo testicular growth and maturation. Females, which do not appear to keep a stash of food, continue to sleep in, so to speak. They typically don't turn up their body thermostat until about four days before they emerge from their burrows and resume daily aboveground activity.

In their high-latitude habitats, arctic ground squirrels experience conditions that are far more severe in winter compared with those experienced by temperate or alpine-dwelling hibernators. Temperatures within their burrows average 16 degrees during the winter, and fall as low as –10 degrees. Furthermore, light conditions in the mid-Arctic, where these animals live, are not like other places. The midnight Sun shines at the height of summer, while in the depths of winter the Sun stays below the horizon for months at a time.

We were interested in understanding the role that circadian clocks play in this unique environment. An organism's circadian clock is the biochemical system that permits coordination of such daily physiological and metabolic processes as sleeping and eating with changes in the hours of daylight. What role, if any, do circadian clocks play in hibernation? And does an organism go "off the clock" when there are long intervals of constant light or constant darkness? How to tease apart this information from small animals in the wild, which may be burrowed underground or scurrying over the landscape, presented a challenge.

In mammals, the circadian master clock is in the suprachiasmatic nuclei of the hypothalamus in the brain; it oscillates on a twenty-four-hour cycle, keeping very accurate time when it receives daily updates on changes in hours of daylight. It transmits its rhythmic information to oscillators in other brain regions and to peripheral organs, in fact to all organs, by means of a variety of outputs, including body temperature rhythms.

The function of such clocks when environments are effectively constant is debated. In Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and ptarmigan (*Lagopus mutus hyperboreus*)—species indigenous to the High Arctic—animals show short alternating bouts of activity and feeding, and circadian rhythms are absent during seasonal intervals of constant light or constant dark. The team that reported that finding in 2005, led by Bob E. H. van Oort of Norway's Center for International Climate and Environmental Research–Oslo (CICERO), proposed that pattern was common to all polar vertebrates.

Other researchers, however, have found evidence that daily rhythms of activity and physiology are maintained throughout the Arctic summer in a number of invertebrates, and in a variety of vertebrates, including fish, migratory birds, and some mammals. Similarly, we have found that arctic ground squirrels exhibit persistent circadian rhythms of activity and body temperature during summer intervals of constant sun at mid-Arctic latitudes.

The question regarding the status of circadian clocks during hibernation has also perplexed scientists. Earlier research on captive golden-mantled ground squirrels (*Callospermophilus lateralis*) from western North America revealed they have persistent circadian body temperature rhythms during steady-state torpor, though these rhythms were very low amplitude and may have been driven by ambient temperature changes in the environmental chambers in which they were housed. The question is, would we also see evidence for a functional circadian clock when measuring body temperature in free-living arctic ground squirrels?

We studied arctic ground squirrels near the University of Alaska Fairbanks Toolik Field Station on the North Slope of Alaska. In the spring of 2010, we implanted nine adult male ground squirrels with abdominal loggers programmed to record body temperature at thirty-four-minute intervals for up to twenty-four months; we also implanted one juvenile squirrel on August 9 with a smaller abdominal logger programmed to record core body temperature every three and a half hours for up to twenty-four months. In September 2010, each squirrel was recaptured, anesthetized, and outfitted with a temperature and light logger affixed to a neck collar made from zip ties with shrink tubing used to prevent abrasion. The light loggers enabled us to record when the squirrels moved in and out of their burrows and thus follow their daily aboveground activity patterns. In addition we could look for the connection between their exposure to light and the setting and resetting of circadian rhythms. Such very lightweight light meters were originally developed to record time of dawn and dusk experienced by migrating birds, as a means to determine the latitudes of their changing locations.

In the spring of 2011, we successfully recaptured nine of the ten squirrels (one adult was not recaptured despite extensive trapping effort). Of the recaptured squirrels, one had lost its collar, one light logger failed to record, and the implanted body temperature logger of a third squirrel failed. Altogether, we obtained complete body temperature and light exposure data from six squirrels during fall entry into hibernation and heterothermy, those alternating bouts of torpor and active temperature. For four of them we also obtained complete data during the termination of heterothermy and spring emergence (on the other two squirrels, the batteries of the light loggers failed during mid-hibernation).

In the fall, before they entered hibernation, core body temperature in free-living male ground squirrels showed robust daily rhythms, rising 2.4 to 4.8 degrees Fahrenheit during the daytime. Indeed, the animals typically had an anticipatory increase in body temperature that varied from 1.2 to 2.4 degrees even before emerging from their burrows each day and being exposed to light. Daily decreases in body temperature occurred after animals returned to their dark burrows.

Of the six adult male ground squirrels from which we recovered simultaneous body temperature and light exposure data in full, five continued to maintain daytime activity patterns after their release for three to nine days before sequestering themselves in their burrows between September 22 and September 30. These animals subsequently first entered torpor anywhere from two to thirteen days later (on average, 6.6 days later). The sixth squirrel entered his burrow on September 29 and maintained warm (euthermic) body temperature levels for twenty-two days thereafter, during which he occasionally made brief forays aboveground and was regularly exposed to low-intensity light. Despite the limited time spent aboveground, he maintained twenty-four-hour body temperature rhythms. In all six animals, daily peaks in body temperature were lower, and mean body temperature was reduced when animals remained in their burrows and had no aboveground activity.

Minimum body temperature during torpor steadily decreased from October until late December, at which point the soil froze and squirrels began maintaining body temperature that was on average a little below the freezing point of water, but warmer than the surrounding soil. During steady state torpor, body temperature was constant and arrhythmic for as long as thirteen days. Light was never detected by animal-borne light loggers during heterothermy.

Collar temperature closely tracked body temperature (within 2.2 degrees) during torpor and during the intervals of euthermic arousal, but deviated frequently from body temperature after heterothermy had ended and squirrels were euthermic,

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feeding on their food caches belowground. The sensor was not directly against the squirrels' skin and was thus influenced by exposure to ambient conditions when the animals were not curled in a ball within their nests.

In the spring, after ending heterothermy, the four male arctic ground squirrels for which we had complete data remained belowground for twenty-two to twenty-six days before commencing daily activity on the surface, as revealed by resumption of their regular exposure to light. In none of them did we detect significant circadian rhythms during the first ten belowground days of euthermy, suggesting that the generation of circadian body temperature rhythms had ceased at some point during heterothermy.

In general, twenty-four-hour rhythms were restored once animals resumed aboveground activity and were directly exposed to the external solar cycles. But three of four squirrels were exposed to very low-amplitude light four to twenty-three days before they initiated daily aboveground activity, and in one of these we did find that a body temperature rhythm recommenced before resumption of aboveground activity. In that case the period of the rhythm was shorter (about twenty-two hours) than a circadian rhythm that is entrained to a light-dark cycle.

Although we lack data on snow cover during this time frame, snow might account for those low-amplitude light exposures. Snow cover at the study site is intermittent and patchy during the winter months, and squirrels may have been exposed to light coming through the snow pack and into their burrow entrances. Indeed, even below ten inches of snow, light could have filtered into burrow entrances.

If the low body temperature associated with torpor in an arctic ground squirrel prevents RNA transcription and translation within the suprachiasmatic nuclei, then we anticipate the animal's master clock would stop and restart multiple times during hibernation. It would stop during torpor and restart during the arousal episodes, through some fourteen to sixteen cycles over the hibernation season, depending on the animal's sex and age. Such start-stop cycles might lead to desynchrony of individual oscillators within the master clock, thus requiring an external trigger to reset a circadian rhythm.

We cannot discount the possibility, however, that oscillators within the suprachiasmatic nuclei are synchronized and rhythmic throughout hibernation. It could be that the output pathways responsible for the generation of body temperature rhythms are inhibited for some time following the completion of heterothermy.

Likewise, our results are not inconsistent with a hypothesis recently put forward by André Malan at the Institute of Cellular and Integrative Neurosciences, CNRS (National Center for Scientific Research) and the University of Strasbourg, France. He proposes that torpor-arousal cycles are controlled by a circadian clock located in a region other than the suprachiasmatic nuclei, a clock not involved in the generation of body temperature rhythms.

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