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Forum

Ground squirrels – experts in thermoregulatory adaptation

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Ground squirrels exemplify one of the most extreme forms of mammalian hibernation and a convenient model for studying its mechanisms. Their thermoregulatory system demonstrates remarkable adaptive capabilities by maintaining optimal levels of body temperature both in active and hibernation states. Here, we review recent findings and unresolved issues regarding the neural mechanisms of body temperature control in ground squirrels.

Ground squirrels are small terrestrial mammals from the rodent family *Sciuridae*. Most of the ground squirrel species living in colder climates undergo seasonal hibernation as an adaptation for survival at low ambient temperatures and with limited food supply [1]. Hibernation season encompasses prolonged bouts of torpor interrupted by brief interbout arousals (IBAs). During torpor, ground squirrels cease activity and dramatically decrease metabolism, while their body temperature approaches ambient levels. The state of torpor greatly reduces energy expenditure in unfavorable environmental conditions. During IBA, ground squirrels briefly regain close-to-active levels of metabolism and body temperature. Research into the neural mechanisms enabling this extreme form of adaptation has been gaining momentum due to its substantial value for both basic science and translational applications. Several species of ground squirrels, most notably the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), the arctic ground squirrel (*Urocitellus parryii*), and the California ground squirrel (*Otospermophilus beecheyi*), have emerged as especially convenient and widely used animal models for the study of hibernation.

Thermoregulation is a particularly striking aspect of the hibernation phenotype. The thermoregulatory system of ground squirrels faces additional challenges compared to that of nonhibernating mammals (Figure 1). In active state, it maintains high stable body temperature around 37°C independently from environmental conditions, similarly to many other mammals, including, for instance, humans and mice. However, the thermoregulatory system during hibernation must remain operational despite low temperatures and keep the body temperature close to ambient to minimize energy expenditure. Furthermore, it should be able to rapidly switch between the two different modes during transitions between torpor and IBA. How the thermoregulatory system of hibernating mammals addresses such challenges is not fully clear, but several recent studies provide valuable insights. Here, we summarize recent findings on the thermoregulation in ground squirrels across their life cycle and outline promising future directions.

In active state, ground squirrels regulate body temperature according to the principles common for all mammals, which have been thoroughly reviewed elsewhere. Among the recent advances in this area has been the identification of the molecular mechanisms of temperature sensitivity in the brain. It has been well established that changes in brain temperature, which are powerful drivers of thermoregulatory responses, are detected by warm- and cold-sensitive neurons in the pre-optic area of the hypothalamus (POA). However, until recently, the molecules responsible for temperature sensitivity of POA neurons remained largely unknown. One recent study in mice reported that the TRPM2 ion channel contributes to the detection of brain warming through synaptic disinhibition of warm-sensitive POA neurons [2]. In another study, the mouse CNGA3 ion channel was found to act as a cold-potentiated temperature sensor that contributes to cold sensitivity of POA neurons [3]. Notably, in contrast to the mouse ortholog, ground squirrel CNGA3 is not potentiated by cold, and the abundance of cold-activated POA neurons in ground squirrels is reduced compared with mice [3]. Interestingly, the primary mammalian peripheral cold sensor TRPM8 also has decreased cold sensitivity in ground squirrels, particularly at temperatures below 20°C, which agrees with reduced cold avoidance in these animals in behavioral tests [4]. These adaptations may support hypothermia during hibernation in ground squirrels, although this hypothesis and related mechanisms require further study.

Despite the similarities in the thermoregulatory system of hibernating and nonhibernating mammals, only the former is capable of profoundly reducing body temperature toward ambient levels at the onset of hibernation. The drop in temperature results from active suppression of metabolism, which is further reduced due to a passive thermodynamic effect of low temperature.

What are the neural mechanisms responsible for suppression of metabolism at the onset of torpor? Addressing this question has been challenging in part due to the lack of experimental tools for monitoring and modulating neural activity in nonstandard animal models such as hibernating mammals. However, several recent studies take advantage of the state-of-the-art

Figure 1. Thirteen-lined ground squirrels demonstrate remarkable thermoregulatory adaptation. In active state (top left), ground squirrels maintain stable body temperature above that of their surroundings, as seen in the infrared image (top right). During hibernation, ground squirrels enter torpor (bottom left) and maintain body temperature 1–2°C above ambient (bottom right). For the infrared image, a torpid squirrel was temporarily moved from its hibernation location with ambient temperature of 4°C to room temperature at 23°C. Figure courtesy of Gracheva lab.

tools available in laboratory mice to make significant progress in this direction. One study focused on the daily torpor response, which refers to the short-term reduction in metabolism and body temperature observed in mice upon fasting [5]. It reported that a population of neurons in the anteroventral portion of the POA, marked by the expression of VGLUT2 and ADCYAP2 genes, was necessary and sufficient for daily torpor induction. Although daily torpor is distinct from hibernation in several important ways, it shares some similarities and may involve common mechanisms. Another pair of studies in mice found that activation of a population of POA neurons marked by the expression of the QRFP gene induced a long-lasting hypothermic and hypometabolic state resembling hibernation, which can be controlled experimentally with high temporal precision through a novel optogenetic method [6,7]. Hypothermia in mice may also be induced by activation of POA neurons that respond to ambient warmth and co-express the BDNF and ADCYAP1 genes [8]. These studies suggest that the molecularly defined populations of hypothalamic neurons may also regulate metabolism and body temperature in ground squirrels. Determining the presence and function of these neuronal populations in hibernators is an exciting area for future research.

What mechanism alters the activity of torpor-inducing neuronal populations as ground squirrels enter hibernation in the fall? A series of studies point to the role of adenosine signaling in this process. Activation of the adenosine A1 receptors (A1ARs) was shown to be necessary and sufficient for the induction of torpor in arctic ground squirrels [9]. Importantly, this effect was present only during the winter season when ground squirrels are naturally primed to begin hibernation, suggesting the sensitization of the adenosine signaling by another circannually regulated factor. This seasonal sensitization is supported by a recent finding that the potency of A1AR agonist increases during the hibernation-permissive season, as measured by the GTP binding assay in hypothalamic tissue from arctic ground squirrels [10]. Importantly, the circannually regulated factor causing the sensitization remains unknown.

Although most vital processes are suspended in hibernating ground squirrels, the animals still respond to external stimuli such as overcooling or warming, and may be quickly aroused from hibernation, regaining normal body temperature within a couple of hours. This reveals another striking property of the thermoregulatory system of ground squirrels – the ability of peripheral neurons to sense thermal stimuli even after being exposed to near-freezing temperatures for several months. This ability may be explained by the recent finding that somatosensory neurons of hibernating ground squirrels remain in a semi-active state: they have altered firing properties, but can generate action potentials upon stimulation [11].

The CNS of hibernating ground squirrels must also retain the ability to invoke heat-generating mechanisms to arouse from torpor. The specific molecular adaptations underlying this ability have not yet been determined. However, intriguingly, a recent study in thirteen-lined ground squirrels found that a large number of genes are differentially expressed across the hibernation cycle, revealing a profound remodeling of the brain transcriptome [12]. Another major achievement of the study was the release of a more precise sequence and annotation of the thirteen-lined ground squirrel genome, which will be instrumental in further characterizing gene expression changes in this species.

In summary, the thermoregulatory system of ground squirrels demonstrates remarkable adaptive capabilities required to cycle between dramatically different physiological states across the hibernation cycle. The complex mechanisms supporting these abilities are still poorly understood, but the growing interest in hibernation research and an expanding set of experimental tools foreshadow significant progress in addressing this gap. A detailed understanding of thermoregulatory adaptation in hibernators will facilitate the development of methods for therapeutic modulation of body temperature in nonhibernating mammals, including humans.

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Declaration of interests

The authors declare no competing interests.

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