ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

Cellular, Molecular, and Physiological Adaptations of Hibernation: The Solution to Environmental Challenges

Sarah M. Mohr,[1,2] Sviatoslav N. Bagriantsev,[1] and Elena O. Gracheva[1,2]

[1]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510, USA; email: slav.bagriantsev@yale.edu

[2]Department of Neuroscience and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA; email: elena.gracheva@yale.edu

Annu. Rev. Cell Dev. Biol. 2020. 36:315–38

First published as a Review in Advance on September 8, 2020

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-012820-095945

Keywords

hibernation, torpor, hypothermia, cold, adaptation, neurophysiology

Abstract

Thriving in times of resource scarcity requires an incredible flexibility of behavioral, physiological, cellular, and molecular functions that must change within a relatively short time. Hibernation is a collection of physiological strategies that allows animals to inhabit inhospitable environments, where they experience extreme thermal challenges and scarcity of food and water. Many different kinds of animals employ hibernation, and there is a spectrum of hibernation phenotypes. Here, we focus on obligatory mammalian hibernators to identify the unique challenges they face and the adaptations that allow hibernators to overcome them. This includes the cellular and molecular strategies used to combat low environmental and body temperatures and lack of food and water. We discuss metabolic, neuronal, and hormonal cues that regulate hibernation and how they are thought to be coordinated by internal clocks. Last, we touch on questions that are left to be addressed in the field of hibernation research. Studies from the last century and more recent work reveal that hibernation is not simply a passive reduction in body temperature and vital parameters but rather an active process seasonally regulated at the molecular, cellular, and organismal levels.

Contents

THE HISTORY OF HIBERNATION

Hibernation [see also *hybernation* in the *Oxford English Dictionary* (Oxf. Univ. Press 2020)] comes from the Latin *hibernare*, meaning "to pass the winter." The first recorded use of *hibernation* dates to the Greek philosopher Aristotle (350 BCE), who observed animals such as mollusks, insects, fish, birds, and warm-blooded quadrupeds that "concealed" themselves "out of the way" during winter and often did not touch food. Three hundred years later, Roman naturalist Pliny the Elder also described animals that "passed the time [of winter] in sleep" (Pliny 77 CE). Experimental investigation into hibernation commenced during the Renaissance era, when pioneering Swiss physiologist Conrad Gessner (1551) described brown adipose tissue in hibernating marmots.

Given the usage of hibernation dates to BCE, the term has been used colloquially and imprecisely over the years. Even modern-day publications confusingly interchange many terms relating to hibernation, including torpidity and hypothermia. For this reason, we define these terms in the context of this review.

WHAT IS HIBERNATION?

Mammalian hibernation refers to a physiological state during which animals repeatedly enter bouts of torpor interspersed with brief periods of interbout arousals (IBAs) (**Figure 1**). Torpor is characterized by regulated core body temperature (CBT) adjustment toward environmental temperatures; decreased heart, respiration, and metabolic rates; decreased blood pressure; and overall activity. An IBA is a temporary state where CBT, heart and respiration rates, blood pressure, and animal activity return to euthermic, or nonhibernating, active values. Although the reason for IBA existence continues to be unclear, it appears to be an essential part of hibernation physiology, as 70% of energy used during hibernation is spent arousing and rewarming the body during IBA (Wang 1979). Several important processes that occur during IBA, but not during torpor,

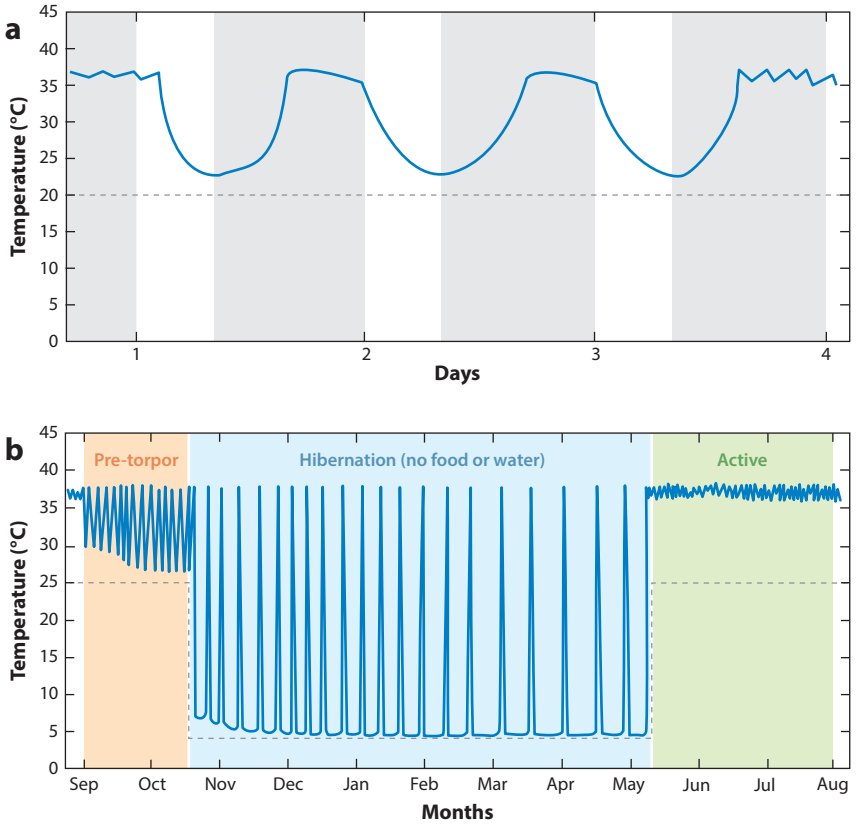

Figure 1

Schematic of core body temperature of (*a*) a daily heterotherm undergoing daily torpor bouts and (*b*) an obligatory hibernator demonstrating seasonal hibernation. The gray dashed lines represent room temperature.

have been identified. These include resumption of transcription, translation, and cell division to produce proteins and cells to replenish and repair old ones; stimulation of the immune system to protect against pathogens; restorative sleep and reversal of dendritic retraction that occurs during torpor; and waste removal from the body (Andrews 2019, van Breukelen & Martin 2015). The length of hibernation, torpor bouts, and IBAs varies by species, size, and geographical range (Ruf & Geiser 2015). For example, thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), which weigh ~200 g and reside at 41° north of the equator, generally hibernate for 5–7 months and experience torpor bouts every 2–3 weeks, interrupted by IBAs lasting ~24 h. Meanwhile, Formosan leaf-nosed bats (*Hipposideros armiger terasensis*), which weigh ~50 g and reside at 17° north of the equator, generally hibernate for 3 months and experience torpor lasting 1 week, interrupted by 2-h-long IBAs.

The term hypothermic has also been commonly used to describe torpid hibernators. Clinically, it refers to an abnormally reduced CBT that is pathologically detrimental to health. Most generally, it can simply refer to a reduction in CBT. Here, we use hypothermia in the latter sense to describe lowering of CBT regardless of hibernation status. Active animals are solely euthermic, with vital parameters and metabolism similar to those of nonhibernators. The set of physiological alterations observed during hibernation is not limited to winter. Some animals living in hot or dry

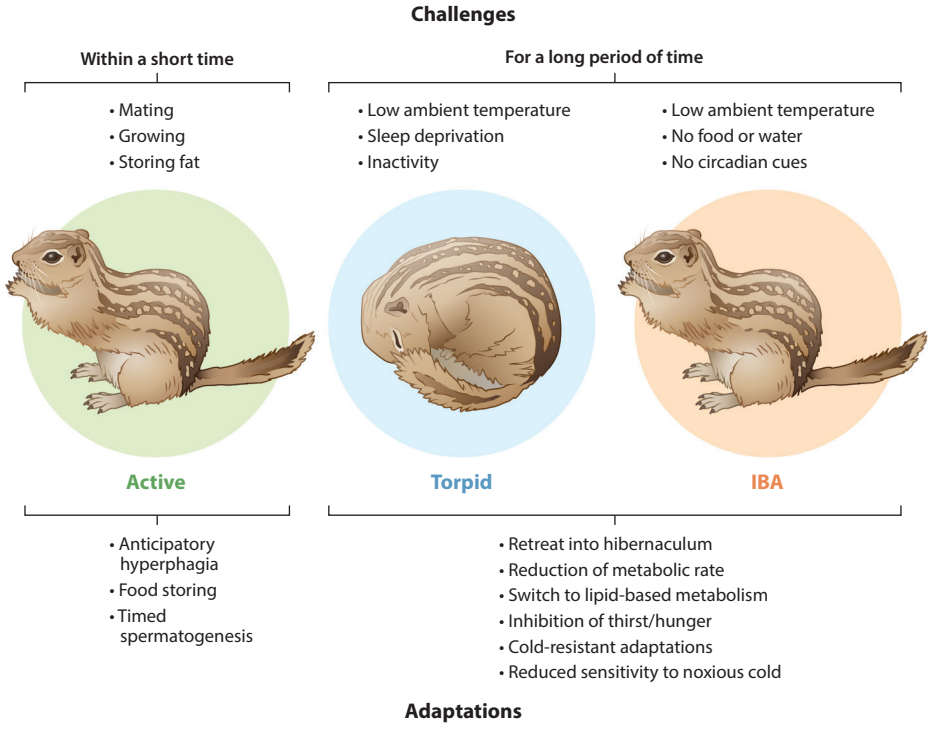

Figure 2

State-specific challenges and adaptations of hibernators. Animals face unique challenges during their active, torpid, and interbout arousal states. They perform anticipatory and acute actions to address these challenges. Abbreviation: IBA, interbout arousal.

environments can estivate during unfavorable conditions and show depression of the same vital signs and metabolism as hibernators (Storey & Storey 1990).

WHICH ANIMALS HIBERNATE AND WHY DO THEY DO IT?

Hibernation, at its root function, employs a collection of physiological strategies that allow animals to inhabit inhospitable environments, where they experience extreme thermal challenges and scarcity of food and water (**Figure 2**). The alternative strategy to hibernation is migration, in which animals relocate to avoid extreme climates in favor of temperate ones with readily available resources. While migrators maintain core physiological parameters and find environments to suit their set points, hibernators instead manipulate integral physiological parameters to stay in one location. This may be one reason why there are few avian hibernators, because many birds are capable of flying thousands of miles to reach more temperate climates.

To go from being an active animal, hunting/foraging, growing, and mating, to being inactive for half a year in a burrow requires immense coordination of different systems that must adjust to hibernating levels at the appropriate time and rate. Despite the challenges of coordinating hibernation, the diversity of animals that demonstrate it is surprisingly vast. Warm-blooded hibernators include mammals (e.g., rodents, marsupials, carnivores, bats, and primates) and one known species of bird (the common poorwill, *Phalaenoptilus nuttallii*) (Lyman et al. 1982). The presence of hibernators among evolutionarily distant clades suggests the adaptation stems from modifications of conserved physiological pathways common to all vertebrates. The most parsimonious hypothesis

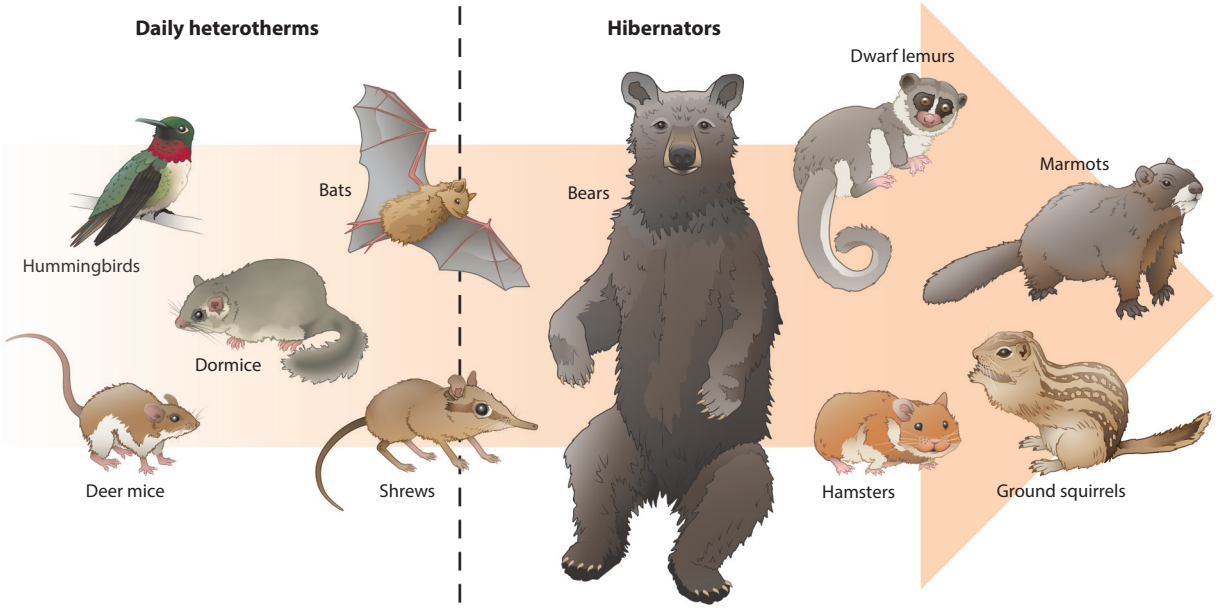

Figure 3

Examples of warm-blooded animals undergoing daily torpor, hibernation, or both. (*Left to right*) Daily torpor: deer mice, hummingbirds, and dormice; both daily torpor and hibernation: shrews and bats; hibernation: bears, dwarf lemurs, hamsters, marmots, and ground squirrels.

for the evolution of hibernation is that it is plesiomorphic. That is, the ability to enter torpor during hibernation is a remnant of ancestral heterothermy that has been retained in some animals, whereas homeothermy was adapted by others (Geiser 2008, Grigg et al. 2004, Lovegrove 2012).

There is a wide variety of hibernation and heterothermic phenotypes (**Figure 3**). Daily heterotherms enter torpor during their natural inactive period and follow a circadian cycle, more similar to deep sleep than hibernation. Daily heterothermy, or daily torpor, is highly prevalent in many species of birds, small rodents, marsupials, and bats (Ruf & Geiser 2015). Other animals, termed facultative hibernators, enter torpor when resources are scarce, temperatures are cold, and changes in photoperiod forebode changes in the season (Folk 1974, Harlow 1997). Facultative hibernators include small- and medium-sized rodents such as hamsters, as well as some marsupials and bats. Still other animals, termed obligatory hibernators, enter torpor seasonally, regardless of resource availability, environmental temperature, and photoperiod (Davis 1976, Folk 1974). Hibernation phenotypes can also be classified by depth, or the general reduction of key physiological parameters described above. Characteristics of hibernation and torpor patterns can be attributed to taxonomy, geographical range, body size, social versus solitary living, and energetic demands of mating and growth. Given the variety of heterothermic phenotypes, hibernators and daily heterotherms likely utilize different mechanisms to regulate torpor. Therefore, it should also not be assumed that all animals use some extension of a central hibernation physiology.

Given the diversity of animals and variety of hibernation strategies they employ, this review focuses on obligatory mammalian hibernators. We identify unique challenges these animals face and explain what is known about adaptations that allow hibernators to overcome them. This includes cellular and molecular strategies used to combat low environmental and body temperatures and lack of food, water, and sleep. We discuss metabolic, neuronal, and hormonal cues important

Table 1 Core physiological parameters of mammalian sleep, daily torpor, and hibernation[a]

	Sleep	Daily heterothermy	Hibernation	
			Shallow torpor	*Deep torpor*
Minimum body temperature (°C)	35–37°C	5–30°C	10–30°C	−3–10°C
Respiration rate (% of active)	100–80%	5–20%	84%	2–3%
Heart rate (% of active)	70–90%	10–30%	10–50%	1–4%
Metabolic rate (% of BMR)	70–90%	5–70%	10–20%	1–10%
Blood pressure (% of active)	85%	60–70%	20–60%	
Daily activity level	High	High	Low	None
Average energy savings	5–15%	60–70%	Up to 90%	
Duration	<24 h	<24 h	>24 h	>24 h
Occurrence	Year-round	Year-round	Season inducible	Seasonal

[a]Shading refers to magnitude of parameter (i.e., more intense color represents higher body temperature or longer duration of time).
Abbreviation: BMR, basal metabolic rate.

for regulating hibernation and their coordination by circadian and circannual rhythms. Last, we touch on questions left to be addressed in the field of hibernation research.

WHAT IS OBLIGATORY HIBERNATION?

Mammalian, obligatory hibernators undergo seasonal hibernation despite favorable conditions, including mild ambient temperatures and plentiful access to shelter and resources, and without changes in photoperiod. Because obligatory hibernation is robust to most environmental manipulations, it is thought to be coordinated by an internal seasonal clock. Obligatory hibernators include several members of the *Sciuridae* family, including species of ground squirrels, white-tailed prairie dogs (*Cynomys leucurus*), and marmots; some species of the primate genus *Cheirogaleus*, known as dwarf lemurs; European hedgehogs (*Erinaceus europaeus*); and European badgers (*Meles meles*). Of these, the deep hibernators live in the most extreme environments and remain in hibernation for 5–7 months. During torpor, animal CBT drops to near ambient levels, although it is defended from dropping far below 0°C. This is necessary in climates of Alaska, Siberia, Western Canada, and the Great Lakes region of North America, where ambient temperatures, even in insulated burrows, can drop below 0°C. For example, the Arctic ground squirrel (*Spermophilus parryii*) in Alaska can drop its CBT to below freezing but defends it against a decrease below −3°C. Hypothermia is also associated with a reduction in heart and respiration rates, which drop to 3–10% of euthermic values (Andrews 2019). Blood pressure is reduced to 20–60% of euthermic values (Horwitz et al. 2013, Lyman & O'Brien 1963), and metabolism is reduced to 1–10% of basal metabolic rates (Barnes & Ritter 1993, Buck & Barnes 2000, Geiser & Kenagy 1988, Geiser & Ruf 1995, Heldmaier et al. 2004) (**Table 1**). During IBA, when animals temporarily enter an active-like state, feeding and drinking either cease or are dramatically reduced. Physiology and animal behavior during hibernation are driven by fundamental differences in the function of cellular and molecular processes in the cold. For example, transcription and translation are halted at temperatures that obligatory hibernators' bodies experience during torpor. Therefore, the survival of obligatory hibernators depends on important adaptations to work at the limitations of basic biology and physiology.

HOW TO GET ENOUGH SLEEP AND RETAIN MEMORIES

Hibernation has historically been likened to sleep. Indeed, sleep and entrance into torpor are endogenously regulated and similar parameters decrease during both: activity, CBT, blood

pressure, and heart and respiration rates (**Table 1**). It may be tempting to think of torpor as deep sleep. Early evidence suggests sleep does not occur during torpor, and animals may become sleep deprived during the process. Mammalian sleep can be described by two main phases: rapid eye movement (REM) and slow wave sleep (SWS). During early entrance into torpor (CBT of 35 to 25°C), cortical electroencephalography (EEG) in ground squirrels measures slow wave activity (SWA) of 1 to 4 Hz, suggesting sleep may be permissive of hibernation entrance. As CBT further decreases to 25–10°C, signal amplitude decreases, the width of spikes increases, and there is a shift toward cycles of 4 to 8 Hz. During deep torpor, below 10°C, brains are isoelectric. When squirrels transition to IBA, they immediately enter what appears to be SWS. Post-torpor SWA intensity increases with torpor length and decreases during IBA (Daan et al. 1991, Walker et al. 1977), suggesting sleep deprivation is accumulated during torpor and resolved during IBA.

However, more recent studies question post-torpor SWA as an indicator of sleep deprivation. If animals are prevented from sleeping upon entrance into IBA, the originally observed SWA indicative of sleep deprivation is eliminated (Larkin & Heller 1999, Strijkstra & Daan 1998). Djungarian hamsters (*Phodopus sungorus*), which experience daily torpor, show similar SWA even though less sleep deficit is built up (Deboer & Tobler 2003). Furthermore, EEG correlates characterizing resolution of sleep deprivation induced during the main phase of IBA differ from those characterizing resolution of post-torpor SWA (Strijkstra & Daan 1998). Therefore, post-torpor SWA may not be an indicator of sleep deprivation but rather a period of synaptogenesis following reversible loss of dendritic complexity during torpor (e.g., Popov et al. 2007, von der Ohe et al. 2006), where SWA reflects tonic hyperpolarization of cortical neurons until dendritic connections are reformed (reviewed in Heller & Ruby 2004, Roth et al. 2010). Sleep occurs during the main phase of IBA and likely is an important reason why it exists. The quality of sleep may also be temperature and species dependent, with some species of primates showing exclusively SWS or REM sleep (Blanco et al. 2016, Krystal et al. 2013). Therefore, although sleep does not occur during deep torpor, its homologous functions may be permissive for entrance into it.

Whether animals retain memories during hibernation is still controversial. Studies of ground squirrels suggest, upon spring emergence, animals have reduced spatial, operant, and social memory (Mateo & Johnston 2000, Millesi et al. 2001), while studies of alpine marmots and bats suggest long-term memory of operant conditioning and spatial tasks is not impaired (Clemens et al. 2009, Ruczynski & Siemers 2011). The time it takes marmots to learn a task taught in the spring is decreased, hinting at a seasonal dependence of learning ability in this species (Clemens et al. 2009). Indeed, dynamic changes in the nervous system of hibernators, including increased dendritic lengths, arborization, number of dendritic spines, and synapse size, are demonstrated during spring emergence (Ruediger et al. 2007; Strijkstra et al. 2003; von der Ohe et al. 2006, 2007). Given the importance of sleep, including memory consolidation, immune function, energy conservation, and stress responses (Roth et al. 2010, Sharma & Kavuru 2010), its role during IBA holds importance for further investigation.

HOW TO ANTICIPATE THE SEASONS: CIRCANNUAL RHYTHMS

Hibernation adapted over time to address ecological challenges presented by seasonal changes. For facultative hibernators, environmental factors (zeitgebers) such as change in photoperiod, scarcity of food, and cold are sufficient to induce torpor. In contrast, for obligate hibernators, torpor is not acutely induced by these factors but instead is regulated by an endogenous circannual clock. A rhythmic process is circannually regulated if it persists under constant environmental conditions for a period of approximately 1 year, is entrained by some zeitgeber, and responds differentially to the zeitgeber at different phases of the cycle (Davis 1976). Circannual clock-keeping is not limited to regulating immergence into and emergence from hibernation but also includes food and

water intake, metabolism, endocrine activity, and gonadal development (Armitage & Shulenberger 1972). These processes occur at the expected time of year even when environmental conditions are kept constant (Davis 1976, Kenagy 1980). In grounds squirrels, the circannual cycle is approximately 11 months (Kenagy 1980). How the circannual clock is entrained, the timescale of entrainment, and the zeitgeber(s) are currently unknown. Many hypotheses have been proposed, including frequency demultiplication, or counting of circadian days; tissue-autonomous cyclic histogenesis, in which local ontogenetic life–death programs inform seasonal cellular function; identification of a sequence of physiological processes such as changes in hormone and enzyme production; an unidentified molecular circannual or torpor-arousal clock; and transcription-independent timekeeping. However, these hypotheses are speculative or unproven for hibernators (Hazlerigg & Lincoln 2011, Malan 2010, Reddy & Rey 2014, Wikelski et al. 2008).

The earliest and most well-known hypothesis of circannual clock regulation is frequency demultiplication of circadian cycles, in which superharmonics of circadian cycles entrained during the active season create phases of the circannual cycle that allow animals to keep yearly time during hibernation, when animals are sequestered in their burrows and do not have access to photic cues (Gwinner 1986). One study of ground squirrels kept in artificially constant photoperiods for 3 years found circannual regulation of body weight, gonadal development, and water consumption are lengthened (Lee & Zucker 1991). This finding implies photoperiod may act as a zeitgeber that requires multiple years for entrainment. However, studies performed with laboratory-born ground squirrels kept at constant but lower temperatures for 4 years found no such changes (Pengelley & Fisher 1963, Pengelley et al. 1976), arguing against the frequency demultiplication hypothesis. The effect of photoperiod on entrainment of circannual rhythms therefore is weak and likely occurs prior to hibernation. For juveniles, this can be only a few months of daylight. Given the necessity of at least 1 year of altered photoperiod to change circannual rhythms in the laboratory, at least in adult animals, there are likely other zeitgebers that play a role or there may be a privileged developmental stage during which entrainment occurs. Indeed, hibernators do not experience health problems associated with altered photoperiod, including dysregulation of the immune system and metabolism, arguing against the singular importance of photoperiod (Labrecque & Cermakian 2015, Marcheva et al. 2013, Maury 2019). In line with this, there is some variation in natural hibernation correlated with latitudinal and altitudinal clines, genetic components, or both, implying there is an environmental effect on circannual regulation in obligate hibernators (Grabek et al. 2019, Williams et al. 2014).

In search for a central regulator of circannual rhythmicity, researchers have proposed the suprachiasmatic nucleus (SCN) of the hypothalamus, the site of circadian regulation, is also involved in maintaining circannual rhythms. However, studies that lesioned the SCN in obligate hibernators found yearly cycles of weight gain, food consumption, and metabolism are mainly unaltered (Zucker et al. 1983). Further studies of SCN-lesioned animals found increased intergroup variability as well as a temperature dependence of the hibernation cycle of some ground squirrels (Ruby et al. 1998, Zucker et al. 1983). Analysis of neuronal activation by c-fos in situ hybridization revealed increased messenger RNA (mRNA) in the SCN during IBA in ground squirrels (Bitting et al. 1994). Overall, it appears the SCN is not essential, although it may be involved, in the entrainment and progression of circannual rhythms. There are other possible neuronal players responsible for endogenous regulation of the circannual clock. The circumventricular organs are not protected by the blood–brain barrier, whereas other regions of the hypothalamus have privileged access to the blood through fenestrated capillaries. These brain areas have access to circulation and therefore hold potential as integrators of peripheral cues responsible for orchestrating the circannual cycle. Neuroendocrine signals, such as thyroid hormones and melatonin, and metabolites, such as adenosine, have been hypothesized to play important roles in seasonal regulation in

facultative hibernators and may participate in circannual cycles of obligatory hibernators (Coomans et al. 2015, Dardente et al. 2014, Lewis & Ebling 2017, Olson et al. 2013, Sáenz de Miera et al. 2014, Schwartz & Andrews 2013). Tanycytes, radial glial cells that line the third ventricle and send projections into the hypothalamus, have been identified as important players in regulation of endogenous rhythms in hibernators (Lewis & Ebling 2017). Tanycytes detect metabolites and endocrine signals from the periphery and also regulate transport of such components from the blood into the brain (Langlet et al. 2013). Integration between metabolic, endocrine, and circadian cues in the hypothalamus may prove necessary for entrainment and regulation of circannual rhythms.

Although most focus has been placed on finding a central regulator of circannual rhythms, an intriguing speculation is the cyclic histogenesis hypothesis, in which tissue-autonomous life–death cycles induce seasonal physiology (Hazlerigg & Lincoln 2011). The proposed mechanism is governed by an ontogenetic program in which progenitor cells of tissues synchronously initiate cellular division, proliferation, migration, and differentiation to produce mature cells in time with seasonal changes. This putative process could occur in key tissues such as the thyroid, gonads, adrenal glands, and the hypothalamus and could further interact with central and hormonal cues (Hazlerigg & Lincoln 2011). Uncovering the mechanism(s) of circannual regulation in hibernators will require creativity and investigation at multiple levels in both the brain and the periphery, as many of the hypotheses put forth may not be mutually exclusive.

HOW TO NOT FEEL HUNGRY WITHOUT EATING

Mammalian obligatory hibernators can endure 5–7 months without eating. How do animals not feel hungry during hibernation? Obligatory hibernators exhibit seasonal differences in feeding. During summer, they eat voraciously, doubling or tripling their food intake to regain weight lost during hibernation, and preferentially feed on high polyunsaturated fatty acid–containing grains thought to supply essential nutrients for hibernation (Florant et al. 1993, Frank 1994, Geiser & Kenagy 1987, Mrosovsky & Boshes 1986). They continue to generate energy stores throughout the late summer to fall, during the fattening period, in the form of white adipose tissue (WAT). During 2–3 months, wild Arctic ground squirrels increase their fat content seven- to eightfold and golden-mantled ground squirrels (*Callospermophilus lateralis*) can double their body weight (Mrosovsky 1975, Sheriff et al. 2013). Given the importance of obtaining appropriate energy stores to last the winter, an interesting phenomenon is a period of anorexia that occurs a few weeks before hibernation (Schwartz et al. 2015b). During this period, animals show a dramatic reduction in feeding, even if food is plentiful. It is not well understood why and how this elimination of hunger drive occurs. Indeed, such active suppression of appetite appears counterproductive for hibernators, as it wastes time and resources that could be put toward fattening. During hibernation, animals demonstrate little to no feeding, supporting seasonal inhibition of hunger (Healy et al. 2011, Mrosovsky & Boshes 1986).

Feeding regulation in mammals occurs through interactions between peripheral hunger/satiety signals and the hypothalamus. The arcuate nucleus (ARC) has privileged access to blood through which it detects hunger/satiety and energy balance signals produced in peripheral organs. Downstream integrative hypothalamic regions, most importantly the paraventricular nucleus, produce the appropriate feed or stop feeding signals in response to ARC input (Gao & Horvath 2007, Waterson & Horvath 2015). Leptin, produced in WAT, and insulin, produced in pancreatic B cells, are two satiety signals that bind to receptors in the ARC to reduce feeding (Friedman & Halaas 1998, Huang et al. 1996, Niswender & Schwartz 2003). Adiponectin, another hormone produced in WAT, plays a role in insulin sensitization and encourages energy expenditure by acting on hypothalamic targets (Berg et al. 2001, Qi et al. 2004).

During the anorexic period preceding hibernation, thirteen-lined ground squirrels exhibit increased hypothalamic RNA for leptin receptor (LEPR), insulin receptor 2 (ISR2), adiponectin receptor 2 (ADIPOR2), thyrotropin-releasing hormone (TRH), and the growth factor VGF (Schwartz et al. 2015b). TRH and VGF are indicative of a positive energy balance, that is, having an excess of energy stored either as fat or otherwise, and could be involved in orchestrating prehibernation anorexia (Al-Arabi & Andrews 2005, Jethwa et al. 2007, Steward et al. 2003). In support of transcriptomic data, the concentration of leptin in serum in woodchucks (Concannon et al. 2001, Florant et al. 2004, Schwartz et al. 2015b) and ground squirrels (Chen et al. 2008, Schwartz et al. 2015b, Xing et al. 2015) increases during the active season as fat mass increases, reaching a yearly peak concentration after the animals have started fall anorexia. Insulin levels are highest at peak fat mass during the active season (Boswell et al. 1994). The level of adiponectin mRNA in WAT is inversely correlated with fat mass (Florant et al. 2004). Infusion of leptin (Boyer et al. 1997, Ormseth et al. 1996, Xing et al. 2016) and insulin (Boswell et al. 1994, Florant et al. 1991) in active ground squirrels decreases feeding, which makes them important candidates to trigger prehibernation anorexia. A better understanding of changes in the sensitivity and regulation of circulating energy balance cues is also essential for understanding different seasonal responses to the same signal. For example, seasonally distinct feeding responses to insulin are demonstrated (Florant et al. 1991). Additionally, in obese individuals, leptin and insulin resistance are reported (Banks et al. 2004, Kahn et al. 2006). Given that hibernators demonstrate increased adiposity during the fattening period, these processes may be involved in seasonal feeding regulation (Florant et al. 1985, Wu et al. 2013).

Hunger cues in hibernators have also been investigated. Plasma ghrelin, a hunger hormone produced in the stomach that acts on the ARC (Betley et al. 2015, Kojima et al. 1999), is increased during spring emergence (Healy et al. 2010). Injection of ghrelin and neuropeptide Y (NPY), a potent orexigenic modulator with actions on the hypothalamus (Clark et al. 1984, Stanley & Leibowitz 1984), increases feeding in ground squirrels (Boswell et al. 1993, Healy et al. 2011). In addition, expression of NPY and agouti-related protein, a neuropeptide and marker of a subpopulation of ARC neurons responsible for hunger (Hahn et al. 1998, Ollmann et al. 1997), is upregulated during spring emergence (Schwartz et al. 2013). The above studies suggest these neuropeptides and the neurocircuitry underlying feeding behavior may function similarly to those in nonhibernators, but further investigation is warranted across seasons (Blouet & Schwartz 2012, Florant & Healy 2012).

Coordination of hunger and satiety is essential for animal survival, as premature emergence from the hibernacula to seek food may dysregulate other dependent processes and increase the risk of predation (Turbill et al. 2011). Although food is not necessary during hibernation, some obligatory hibernators cache food in their burrows. However, it is not clear whether this food is consumed during IBAs or meant for consumption during spring, when the animal can benefit from extra energy to emerge from their burrows, gather food, and find mates. If the latter is true, then hunger suppression during hibernation would be an important mechanism that prevents animals from depleting food stores before spring.

HOW TO SUPPLY THE BODY WITH ENERGY: A SWITCH IN METABOLISM

One of the central concepts behind hibernation is energy conservation during resource scarcity. It is an adaptive trade-off that outweighs the risk of predation in a decelerated state. Dramatic reduction in metabolic rate is achieved through temporary reduction in CBT and the function of several energy-demanding organs, including the brain (Drew et al. 2007), kidneys (Jani et al. 2013),

liver (Gehnrich & Aprille 1988), digestive system (Carey & Assadi-Porter 2017), and reproductive system (Barnes 1996), as well significant reduction in cell autonomous processes (Andrews 2019).

There is some matter of debate about how CBT and metabolic rate are coupled during torpor entrance. There are three main hypotheses (reviewed in detail in Geiser 2004): (*a*) Temperature and metabolism are reduced simultaneously; (*b*) temperature reduction precedes metabolic reduction; and (*c*) metabolic reduction precedes temperature reduction. Mitochondria are responsible for converting nutrient energy into ATP, and their activity is affected by the Q_{10} effect. The Q_{10} effect states that as the temperature of a reaction system is reduced, there is a passive reduction in the kinetic energy available to fuel the reaction. As such, mitochondrial substrate oxidation is reduced at low temperatures. In ground squirrels, a liver-specific reduction in mitochondrial substrate oxidation is reduced beyond what would be predicted by the Q_{10} effect, implying there is active inhibition of mitochondrial function (Barger et al. 2003, Muleme et al. 2006). Another example of active inhibition of metabolism is demonstrated in hibernating bears, who drop their CBT to only 30–36°C while achieving a metabolic reduction of 75% (Tøien et al. 2011). Thus, factors such as surface area–to–volume ratio of the animal and torpor pattern also influence the uncoupling between metabolism and CBT during torpor entrance.

During hibernation, animals switch from carbohydrate-based to primarily lipid-based metabolism. This strategy is advantageous for hibernators, because more energy can be stored in the form of fat than in the form of glucose and glycogen. The switch to lipid-based metabolism can be measured indirectly through calculations of respiratory quotients (RQs), or the ratio of CO_2 to O_2. Arctic ground squirrels showed an RQ of 0.7 during torpor bouts, indicating lipid metabolism (Buck & Barnes 2000, Lusk 1924). This value is supported by the downregulation of enzymes responsible for carbohydrate metabolism and the upregulation of enzymes responsible for fatty acid β-oxidation during winter (Andrews 2004, Carey et al. 2003, Epperson et al. 2009, Hindle et al. 2011). Additional adaptations inhibit carbohydrate metabolism during hibernation, which may protect carbohydrate stores in case of need. The entry of glycolytic intermediates into the tricarboxylic acid (TCA) cycle is inhibited through upregulation of pyruvate dehydrogenase kinase 4 in the heart, WAT, and skeletal muscle (Buck et al. 2002). Upregulation of pancreatic triglycerol in the heart and WAT during hibernation increases fatty acid liberation for β-oxidation (Squire et al. 2003). Although fatty acids act as the main fuel for much of the body, some tissues, such as brain and heart, require specific energy substrates. In the liver, fatty acids are converted to ketone bodies, which are readily catabolized by the heart and brain (Andrews et al. 2009). β-Hydroxybutyrate (BHB), one of the endogenous ketone bodies produced from lipid hydrolysis, can be used as a marker of ketone bioavailability. Thirteen-lined ground squirrels show an upregulation in a ketone transporter, monocarboxylic acid transporter 1, and an increase in plasma BHB during torpor and IBA (Andrews et al. 2009, Feng et al. 2019, Regan et al. 2019). Ketone metabolism does not produce damaging lactic acid, a by-product of anaerobic glycolysis, and may protect tissues during hibernation (Andrews et al. 2009). Although in vivo nuclear magnetic resonance of radiolabeled glucose shows glucose is increased in the brain during IBA, few metabolites from the TCA cycle are labeled, indicating glucose is not utilized for ATP production. Instead, high concentrations of TCA cycle metabolites from radiolabeled BHB are found, implying ketones are the primary fuel source for the brain during hibernation (Andrews et al. 2009).

Due to the reliance on lipid metabolism, one may expect blood glucose levels to be lower during hibernation than during the active state. Some groups found serum glucose is lower during torpor than during the active state (Andrews et al. 2009, Galster & Morrison 1975), whereas others report unchanged glucose levels across states (Feng et al. 2019, Regan et al. 2019). Several studies report evidence for both gluconeogenesis and glycogen replenishment in the liver and kidney during IBA (Galster & Morrison 1975, Gehnrich & Aprille 1988, Green et al. 1984), but it is unclear whether

gluconeogenesis occurs during torpor. Sources for gluconeogenesis include glycerol, a by-product of triglyceride hydrolysis, and amino acids obtained from protein breakdown. The breakdown of muscle protein seems to be protected, even despite several months of inactivity (Gao et al. 2012), and suggests if gluconeogenesis occurs, the substrates likely come from triglyceride breakdown. Refreshment of glucose during IBA may serve as an energetic reserve in the case of ketone scarcity or in response to increased metabolic demand, but the exact role is unclear.

Despite the reduction in cellular energy requirements during hibernation, some cell types must retain their function to ensure survival of the animal. Specifically, the nervous system plays an essential role in coordinating torpor–IBA transitions. Although much of the brain is electrically quiescent during torpor, some regions, such as the hypothalamus, must remain active (Bratincsák et al. 2007). The hypothalamus is responsible for performing essential autonomic functions such as thermogenesis, energy and water balance, circadian rhythms, and sleep. Furthermore, the somatosensory system must detect dangerously low ambient temperatures to initiate thermogenic responses, as well as regain normal function upon arousal (Hoffstaetter et al. 2018). Mitochondrial respiration and membrane potential of neurons are higher, and proton leak is lower, in hibernating animals than in spring-active animals, implying hibernating neurons are more efficient at generating energy (Ballinger et al. 2017). Therefore, metabolism requires complex coordination in order to ensure essential tissues and cell types receive the energy required for proper function and maintenance.

HOW TO SURVIVE WITHOUT WATER

Hydration and fluid balance are essential for life. Indeed, water makes up 60–80% of the human body (Masento et al. 2014). Maintenance of proper cellular and blood osmolality, or the concentration of solutes, is necessary to maintain cellular volume and shape, ionic gradients, and transport across membranes. As little as a 1% increase in osmolality produces thirst, while an increase of approximately 3% to 15% results in fatigue, dizziness, and cognitive impairment. Death due to dehydration occurs at a water loss of 15% to 25% (Ashcroft 2000, Bekkevold et al. 2013, Leib et al. 2016, Masento et al. 2014). Reliable water is even more essential than a reliable food source, as humans will die in 3–7 days from dehydration but can survive more than 1 month without food. Given the importance of hydration, thirst has evolved to become an incredibly potent motivational drive.

Obligatory hibernators do not drink while they hibernate over a period of 5 to 7 months (Feng et al. 2019). How are they able to control blood osmolality during this time and withstand the presumably strong thirst drive to seek water? Intriguingly, studies have found plasma osmolality of several hibernators, including thirteen-lined ground squirrels, marmots, and prairie dogs, decreases during torpor by as much as 10% (Bito & Roberts 1974, Feng et al. 2019, Hamilton & Pfeiffer 1977). To maintain the appropriate osmolality, animals can increase drinking in response to, or in anticipation of, activities that would increase osmolality, such as feeding, water deprivation, and water loss. In addition, they can increase water retention. Hibernators do not drink during IBA, even when offered water, and they continue to lose water through respiration and urination. However, they reduce plasma osmolality during torpor. Although rehydration through lipid metabolism has been ruled out, it is still not clear how this drop occurs (Feng et al. 2019). During torpor, water may leave the intracellular compartment, most likely skeletal muscle, because of decreasing concentrations of amino acids and metabolites due to inactivity. Possibly, osmolytes are reabsorbed from the extracellular fluid and sequestered into bodily compartments such as the bladder, as has been proposed for hibernating bears (Spector et al. 2015). The reduction in plasma osmolality during torpor further seems to be actively maintained and reversible, as it increases during IBA to active levels (Feng et al. 2019). When their blood osmolality is artificially raised by

injection of hyperosmotic solutions, IBA ground squirrels increase drinking. These two observations suggest thirst circuitry is inhibited but remains activatable during hibernation.

Another mechanism to enforce low osmolality is to increase water retention. Water retention is achieved through interactions between hypothalamic osmosensors, which detect changes in blood osmolality and produce the feeling of thirst; downstream brain regions, which synthesize and release antidiuretic hormones; and the kidneys, which contain receptors that bind these hormones and promote water retention during dehydration (Bichet 2019, Mandelblat-Cerf et al. 2017, Zimmerman et al. 2017). Antidiuretic hormone release is reduced during torpor, consistent with reduced kidney function reported during torpor (Feng et al. 2019). This also implies ground squirrels are not able to reduce their blood osmolality through increased retention of water and that another mechanism is responsible. During IBA, when osmolality returns to active levels, antidiuretic hormones also return to active levels despite the reduction of thirst. This suggests regulation of thirst and antidiuretic pathways are decoupled during hibernation.

Inhibition of thirst during hibernation has a clear adaptational advantage, as hibernators do not have to emerge from their burrows to seek water and risk predation. However, the mechanisms of reversible osmolyte redistribution during torpor, neural regulation of thirst and antidiuretic hormone release, and their uncoupling during hibernation remain to be elucidated.

HOW TO SURVIVE IN THE COLD

Nonhibernating animals that have a reduction in CBT of just a few degrees Celsius experience moderate hypothermia, associated with shivering and reduced muscle coordination. During profound hypothermia (a reduction of more than 10°C), animals experience massive organ failure due to dysregulation of ionic gradients and changes in membrane fluidity. Integral cellular processes such as transcription and translation are also dramatically reduced. Moreover, these temperatures are perceived as noxious and painful. Shivering to increase CBT is energetically costly and requires an increase in metabolism that depletes energy stores. Even when nonhibernating animals survive hypothermia and regain euthermy, cellular damage and apoptosis occur due to reperfusion injury.

Obligatory hibernators experience near-freezing CBTs for 2–3 weeks, multiple times per season without deleterious effects. What adaptations enable hibernators to survive these conditions? The following section outlines what is known about cellular mechanisms that maintain ionic gradients and the function of thermosensory ion channels. The reader is advised to refer to the following references to learn about other adaptations not discussed here: translation, transcription, and posttranscriptional/posttranslational regulation in the cold (Andrews 2019); thermoregulation (Arnold 1988, Heller et al. 1977, Staples 2016, Staples & Brown 2008); reperfusion injury (Drew et al. 2001); metabolism (see the section titled How to Not Feel Hungry Without Eating); membrane fluidity (Kolomiytseva et al. 2008; Ruf & Arnold 2008; Suri et al. 2012, 2013); and mitochondrial function (Hendriks et al. 2020; Staples 2014, 2016).

Alterations in Resting Membrane Potential: How to Remain Functional in the Cold

Cooling affects many cellular properties, such as membrane fluidity, and the function of membrane proteins, including ion channels and pumps. One molecule susceptible to changes in temperature and critical for the function of all mammalian cells, particularly of excitable nature, is the Na^+/K^+-ATPase. The Na^+/K^+-ATPase maintains appropriate ionic gradients across the cell membrane, influences the resting membrane potential (RMP), preserves cellular shape and volume, and acts as a signal transducer. Cold-induced inhibition of the Na^+/K^+-ATPase

dysregulates ionic gradients across the membrane and leads to intracellular buildup of sodium due to increased passive ion leakage. This results in a more depolarized RMP, which ultimately can lead to excess calcium influx, leading to excitotoxicity and cellular damage. In neurons, in concert with cold-induced lengthening of activation and inactivation of sodium and potassium voltage-gated channels, Na^+/K^+-ATPase inhibition causes increased duration of action potentials, population, and single-unit spikes and reduction in conduction velocity (Krilowicz et al. 1989, Russ & Siemen 1996). Below 14°C, spontaneous activity of neurons ceases completely (Gähwiler et al. 1972, Krilowicz et al. 1989).

Cortical neurons from hibernating hamsters show increased activity of neuronal Na^+/K^+-ATPase at 5–10°C compared with neurons from rats (Goldman & Willis 1973). Increased activity of the pump at low temperatures allows for proper maintenance of ionic gradients and RMP and therefore permits proper excitability. In line with this, RMP is unaltered in hibernating ground squirrels' somatosensory neurons (Hoffstaetter et al. 2018) and muscle cells (Albuquerque et al. 1978) at cold temperatures. Several groups have also found that neurons from hibernators, including ground squirrels and hamsters, conduct action potentials at CBTs below 10°C (Chatfield et al. 1951, Kehl & Morrison 1960, South 1961). Indeed, increased function of Na^+/K^+-ATPase during hibernation may be adaptive for a variety of organs, including the brain (Goldman & Willis 1973), liver, kidney, and muscle (MacDonald & Storey 1999). In some tissues, including nervous tissue, heart, and skeletal muscle, Na^+/K^+-ATPase function is preserved at the same level of activity as that in active hibernators (Guo et al. 2017, MacDonald & Storey 1999), implying molecular modifications in the cell membrane of those tissues or in the Na^+/K^+-ATPase itself allow for proper function in the cold. The state-dependent regulation of Na^+/K^+-ATPase may include reversible phosphorylation, but the exact mechanism remains unknown (MacDonald & Storey 1999).

The function of ion channels, including sodium and potassium voltage-gated channels, is also vulnerable to cold. Changes in activation and inactivation of voltage-gated channels affect voltage dependency, duration, and frequency of action potential firing. Several studies have found action potential duration increases and action potential frequency decreases, but does not cease, in neurons from hibernating ground squirrels (Hoffstaetter et al. 2018, Krilowicz et al. 1989). An early study hints at adaptations in voltage-gated ion channels in cortical neurons, finding a reduction in current density of sodium channels in the paranodal region (Russ & Siemen 1996). The same study found kinetics of activation and inactivation of sodium and potassium channels are similar between neurons from hibernating hamsters and euthermic rats recorded at low temperatures (Russ & Siemen 1996). Channel-specific analysis of somatosensory neurons from hibernating ground squirrels at room temperature demonstrates a partial reduction in density and a depolarizing shift in the voltage dependence of activation of voltage-gated sodium currents responsible for the upstroke of an action potential. Consistent with this finding, such neurons display a reduction in firing frequency (Hoffstaetter et al. 2018). Together, these observations suggest the function of voltage-gated sodium channels is reversibly regulated in hibernators in a state-dependent manner. It remains to be determined whether the observed changes in voltage-gated sodium channel activity underlie the ability of hibernating neurons to preserve firing in the cold. In any case, there are likely additional cellular and molecular mechanisms that contribute to the preservation of neuronal function at low temperatures. Function of neurons and other integral cell types in the cold is essential to allow for rapid arousal upon changing environmental stimuli and regulation of countless other internal regulatory processes. Despite Na^+/K^+-ATPase and ion channel function being integral to mammalian cellular biology, the mechanisms for preserving cellular function and RMP at low temperatures, particularly in specific tissue types, remain to be elucidated. One primary candidate for future investigation is voltage-gated potassium channels, whose action during torpor is essential for the repolarization phase of action potentials.

Alterations in Sensory Channel Activation: How to Not Feel Cold

Most of hibernation occurs at low environmental temperatures, which are perceived as noxious for nonhibernating mammals. In vertebrates, the molecular mechanism of temperature detection by somatosensory neurons involves ion channels of the transient receptor potential (TRP) family. The function of TRP channels is often tailored in accordance with animal physiology and habitat, such that TRP orthologs from different species have variable thermal sensitivities (Hoffstaetter et al. 2018). In mammals, the detection of environmental cold involves TRPM8, a nonselective cation channel expressed in a subset of somatosensory neurons (McKemy et al. 2002, Peier et al. 2002). In rodents, the channel exhibits a progressive activation from 30 to 10°C in vitro and is essential for cold sensitivity in vivo (Bautista et al. 2007, Dhaka et al. 2007). Thirteen-lined ground squirrels and Syrian hamsters (*Mesocricetus auratus*) express an ortholog of TRPM8 with markedly reduced sensitivity to cold (Matos-Cruz et al. 2017). This finding provides a molecular explanation for reduced cold sensitivity of squirrels and hamsters not only during hibernation but also in the active state. A comparison of squirrel and rat TRPM8 revealed that temperature insensitivity of the ground squirrel ortholog results from six amino acid substitutions in the transmembrane core domain of the channel. Notably, these changes are different from those that lead to cold insensitivity of hamster TRPM8, suggesting hamster and ground squirrel orthologs lose cold sensitivity via different mechanisms. In any case, the resultant indifference to low temperatures allows a hibernator to reduce cold sensation that may interrupt behavioral and physiological processes necessary to enter torpor. Altered function of TRPV1, a thermosensory channel expressed in C-type nociceptors activated by noxious temperatures above 42°C (Julius 2013), also has reduced sensitivity in thirteen-lined ground squirrels. The insensitivity of this channel to heat is due to a single amino acid substitution in the N-terminal ankyrin repeat domain (Laursen et al. 2016). Consistent with this, active ground squirrels exhibit reduced sensitivity to noxious heat in behavioral tests. The reductions in TRP channel sensitivity to extreme temperatures were discovered in active animals, suggesting some thermosensory adaptations are constitutively present year-round in hibernators.

Active ground squirrels and hamsters remain sensitive to temperatures below 10°C (Matos-Cruz et al. 2017). The molecular sensor for the low temperature range in mammals is unknown. It is tempting to speculate that such a sensor remains functional in hibernators during the active season to warn of extremely low temperatures. However, during hibernation, when animals are subjected to temperatures close to 0°C, this sensor is expected to undergo a reversible shutdown. Notably, lowering the environmental temperature below 0°C triggers arousal in ground squirrels (Heller 1979), suggesting the existence of yet another unidentified mechanism that protects the animal from freezing during hibernation.

Furthermore, in comparison to mice, thirteen-lined ground squirrels possess fewer cold-sensitive neurons in the preoptic area of the hypothalamus, a key player in thermoregulation. Mouse cold-sensing neurons express CNGA3, a cyclic nucleotide-gated ion channel potentiated by cold, whereas the squirrel ortholog of CNGA3 is cold insensitive (Feketa et al. 2020). Although the physiological function of central thermosensors in homeothermic mammals is not clear, they may be essential for regulating CBT in hibernators, whose brain temperature routinely drops below 15°C (Strumwasser 1958).

Protection of Dynamic Structural Proteins: How to Maintain Structure in the Cold

In the cold (4°C), microtubule-associated proteins can stabilize microtubules for approximately 1 h (Bosc et al. 2003). However, hibernators can remain at 4°C for days without apparent cellular damage. Ground squirrel induced pluripotent stem cell (iPSC) neurons remain healthy and stable

at 4°C (Ou et al. 2018). These neurons retain neurite length and normal levels of tubulin isotypes unlike human and rat iPSC neurons, which show shorter neurites and reduced levels of tubulin isotypes. Further, human iPSC neurons exposed to cold exhibit increased hyperpolarization of the mitochondrial membrane, which causes an increase in reactive oxygen species that damages tubulin. Increased lysosomal membrane permeabilization and reduced expression of heat shock proteins are also hypothesized to damage tubulin stability in rat and human iPSC neurons. Ground squirrel iPSC neurons prove resistant to these stressors, implying they contain mechanisms that are protective against dysregulation of mitochondrial membrane potentials. Mitochondrial uncoupling proteins (UCPs) and protease inhibitors are hypothesized to play a protective role, but this remains to be confirmed. Hibernators may also possess mechanisms to minimize the negative effects of reactive oxygen species, as levels of antioxidants, such as ascorbate and melatonin, increase during hibernation (Drew et al. 2001, Schwartz et al. 2015a). Given the active role of UCPs in brown adipose tissue thermogenesis during hibernation, UCPs may be utilized in other tissues (Laursen et al. 2015) to protect against dysregulated membrane potentials. Although iPSC neurons from ground squirrels seem to be resistant to cold compared with those from nonhibernators at baseline, in vivo reversible alterations in dendritic morphology during torpor–IBA transitions have been observed. Specifically, reduction in arbor and synaptic complexity occurs during torpor, which is reversed within 2 h of returning to euthermy (e.g., Popov et al. 2007, von der Ohe et al. 2006). Neurons isolated from torpid Syrian hamsters show an increased length of the axonal initial segment, suggesting mechanisms may be in place to compensate for the lack of dendritic complexity (León-Espinosa et al. 2018).

THE FUTURE OF THE HIBERNATION FIELD

Although knowledge of hibernation has existed for over two millennia, scientists have only recently developed tools that permit us to intricately probe questions about the complex mechanisms that allow hibernation to occur. Understanding the mechanistic basis of hibernation necessitates the development and application of modern molecular and cellular methods in a wide variety of species. For example, viral delivery of cell type–specific, light-sensitive proteins poses a flexible solution for targeted manipulation of neuronal activity and in vivo imaging, and the possibilities for using clustered regularly interspaced short palindromic repeats (CRISPR)-based approaches to create transgenic animals are yet untapped. Not only must modern tools be used to advance the field, but foundational work must also be performed to create shareable resources for the hibernation community. Organ atlases, neural circuitry tracing, transcriptomes, genomic and proteomic data, and baseline physiological measures of hibernators across physiological states should be performed to allow for cross-species comparisons (Grabek et al. 2015, 2019; Mugahid et al. 2019; Schwartz et al. 2015b). Given that so many different animals are capable of hibernation, comparative studies are integral for discovering central components and modifications surrounding hibernation.

With the development of these new techniques and resources in mind, several fundamental questions remain unanswered. What are the roles of IBA? How is hibernation controlled at the systems level? How does basic cellular function change during hibernation and across torpor–IBA cycles? Which fundamental cellular and molecular changes appear to be universal to all tissues and which are tissue-specific adaptations? Many facets of the immune system, gut microbiome (Carey & Assadi-Porter 2017), reproduction, epithelial barrier permeability, and more are expected to be altered in hibernators and require intense study. These are essential questions to consider as we further push hibernation research to be applied to human health in terms of organ preservation, understanding of obesity and metabolic disease, and induction of torpor for long-term space travel.

The field of hibernation contains many unmined gems of scientific inquiry that deserve to be further investigated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This study was funded by National Institutes of Health (NIH) grant 1R01NS091300-01A1 and National Science Foundation (NSF) award IOS-1754286 to E.O.G. and by NSF grant 1923127 and NIH grant 1R01NS097547-01A1 to S.N.B. We thank Ni Feng and Madeline Junkins for their careful reading of an earlier draft of this review.

LITERATURE CITED

Al-Arabi A, Andrews JF. 2005. The metabolic effect of thyrotropin releasing hormone (TRH) and norepinephrine (NE) in cold-acclimated obese rats. *Biomed. Sci. Instrum.* 41:62–67

Albuquerque EX, Deshpande SS, Guth L. 1978. Physiological properties of the innervated and denervated neuromuscular junction of hibernating and nonhibernating ground squirrels. *Exp. Neurol.* 62(2):347–73

Andrews MT. 2004. Genes controlling the metabolic switch in hibernating mammals. *Biochem. Soc. Trans.* 32(6):1021–24

Andrews MT. 2019. Molecular interactions underpinning the phenotype of hibernation in mammals. *J. Exp. Biol.* 222(2):jeb160606

Andrews MT, Russeth KP, Drewes LR, Henry P-G. 2009. Adaptive mechanisms regulate preferred utilization of ketones in the heart and brain of a hibernating mammal during arousal from torpor. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296(2):R383–93

Aristotle. 350 BCE. *The History of Animals*, transl. D Wentworth Thompson, Book 8, Part 13–17. The Internet Classics Archive. **http://classics.mit.edu/Aristotle/history_anim.mb.txt**

Armitage KB, Shulenberger E. 1972. Evidence for a circannual metabolic cycle in *Citellus tridecemlineatus*, a hibernator. *Comp. Biochem. Physiol. A Physiol.* 42(3):667–88

Arnold W. 1988. Social thermoregulation during hibernation in alpine marmots (*Marmota marmota*). *J. Comp. Physiol. B* 158(2):151–56

Ashcroft F. 2000. *Life at the Extremes*. Berkeley: Univ. Calif. Press

Ballinger MA, Schwartz C, Andrews MT. 2017. Enhanced oxidative capacity of ground squirrel brain mitochondria during hibernation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 312(3):R301–10

Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, et al. 2004. Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes* 53(5):1253–60

Barger JL, Brand MD, Barnes BM, Boyer BB. 2003. Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating Arctic ground squirrels. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284(5):R1306–13

Barnes BM. 1996. Relationships between hibernation and reproduction in male ground squirrels. In *Adaptations to the Cold: Tenth International Hibernation Symposium*, ed. F Geiser, AJ Hulbert, SC Nicol, pp. 71–80. Armidale, Aust.: Univ. N. Engl. Press

Barnes BM, Ritter D. 1993. Patterns of body temperature change in hibernating Arctic ground squirrels. In *Life in the Cold: Ecological, Physiological, and Molecular Mechanisms*, ed. C Carey, GL Florant, BA Wunder, B Horwitz, pp. 119–30. Boulder, CO: Westview Press

Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, et al. 2007. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 448(7150):204–8

Bekkevold CM, Robertson KL, Reinhard MK, Battles AH, Rowland NE. 2013. Dehydration parameters and standards for laboratory mice. *J. Am. Assoc. Lab. Anim. Sci.* 52(3):233–39

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat. Med.* 7(8):947–53

Betley JN, Xu S, Cao ZFH, Gong R, Magnus CJ, et al. 2015. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* 521(7551):180–85

Bichet DG. 2019. Regulation of thirst and vasopressin release. *Annu. Rev. Physiol.* 81:359–73

Bito LZ, Roberts JC. 1974. The effects of hibernation on the chemical composition of cerebrospinal and intraocular fluids, blood plasma and brain tissue of the woodchuck (*Marmota monax*). *Comp. Biochem. Physiol. A Physiol.* 47(1):173–93

Bitting L, Sutin EL, Watson FL, Leard LE, O'Hara BF, et al. 1994. C-*fos* mRNA increases in the ground squirrel suprachiasmatic nucleus during arousal from hibernation. *Neurosci. Lett.* 165(1):117–21

Blanco MB, Dausmann KH, Faherty SL, Klopfer P, Krystal AD, et al. 2016. Hibernation in a primate: Does sleep occur? *R. Soc. Open Sci.* 3(8):160282

Blouet C, Schwartz GJ. 2012. Brainstem nutrient sensing in the nucleus of the solitary tract inhibits feeding. *Cell Metab*. 16(5):579–87

Bosc C, Andrieux A, Job D. 2003. STOP proteins. *Biochemistry* 42(42):12125–32

Boswell T, Richardson RD, Schwartz MW, D'Alessio DA, Woods SC, et al. 1993. NPY and galanin in a hibernator: hypothalamic gene expression and effects on feeding. *Brain Res. Bull.* 32(4):379–84

Boswell T, Woods SC, Kenagy GJ. 1994. Seasonal changes in body mass, insulin, and glucocorticoids of free-living golden-mantled ground squirrels. *Gen. Comp. Endocrinol.* 96(3):339–46

Boyer BB, Ormseth OA, Buck L, Nicolson M, Pelleymounter MA, Barnes BM. 1997. Leptin prevents posthibernation weight gain but does not reduce energy expenditure in Arctic ground squirrels. *Comp. Biochem. Physiol. C* 118(3):405–12

Bratincsák A, McMullen D, Miyake S, Tóth ZE, Hallenbeck JM, Palkovits M. 2007. Spatial and temporal activation of brain regions in hibernation: *c-fos* expression during the hibernation bout in thirteen-lined ground squirrel. *J. Comp. Neurol.* 505(4):443–58

Buck CL, Barnes BM. 2000. Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an Arctic hibernator. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279(1):R255–62

Buck MJ, Squire TL, Andrews MT. 2002. Coordinate expression of the PDK4 gene: a means of regulating fuel selection in a hibernating mammal. *Physiol. Genom.* 8(1):5–13

Carey HV, Andrews MT, Martin SL. 2003. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol. Rev.* 83(4):1153–81

Carey HV, Assadi-Porter FM. 2017. The hibernator microbiome: host-bacterial interactions in an extreme nutritional symbiosis. *Annu. Rev. Nutr.* 37:477–500

Chatfield PO, Lyman CP, Purpura DP. 1951. The effects of temperature on the spontaneous and induced electrical activity in the cerebral cortex of the golden hamster. *Electroencephalogr. Clin. Neurophysiol.* 3(2):225–30

Chen JF, Yang M, Zhong W, Wang D. 2008. Seasonal changes in body mass, serum leptin concentration and UCP1 content in Daurian ground squirrels (*Spermophilus dauricus*). In *Hypometabolism in Animals: Torpor, Hibernation and Cryobiology*, ed. BG Lovegrove, AE McKechnie, pp. 269–80. Pietermaritzburg, S. Afr.: Univ. KwaZulu-Natal Press

Clark JT, Kalra PS, Crowley WR, Kalra SP. 1984. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 115(1):427–29

Clemens LE, Heldmaier G, Exner C. 2009. Keep cool: Memory is retained during hibernation in Alpine marmots. *Physiol. Behav.* 98(1–2):78–84

Concannon P, Levac K, Rawson R, Tennant B, Bensadoun A. 2001. Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281(3):R951–59

Coomans CP, Ramkisoensing A, Meijer JH. 2015. The suprachiasmatic nuclei as a seasonal clock. *Front. Neuroendocrinol.* 37:29–42

Daan S, Barnes BM, Strijkstra AM. 1991. Warming up for sleep? Ground squirrels sleep during arousals from hibernation. *Neurosci. Lett.* 128(2):265–68

Dardente H, Hazlerigg DG, Ebling FJP. 2014. Thyroid hormone and seasonal rhythmicity. *Front. Endocrinol.* 5:19

Davis DE. 1976. Hibernation and circannual rhythms of food consumption in marmots and ground squirrels. *Q. Rev. Biol.* 51(4):477–514

Deboer T, Tobler I. 2003. Sleep regulation in the Djungarian hamster: comparison of the dynamics leading to the slow-wave activity increase after sleep deprivation and daily torpor. *Sleep* 26(5):567–72

Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A. 2007. TRPM8 is required for cold sensation in mice. *Neuron* 54(3):371–78

Drew KL, Buck CL, Barnes BM, Christian SL, Rasley BT, Harris MB. 2007. Central nervous system regulation of mammalian hibernation: implications for metabolic suppression and ischemia tolerance. *J. Neurochem.* 102(6):1713–26

Drew KL, Rice ME, Kuhn TB, Smith MA. 2001. Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. *Free Radic. Biol. Med.* 31(5):563–73

Epperson LE, Rose JC, Carey HV, Martin SL. 2009. Seasonal proteomic changes reveal molecular adaptations to preserve and replenish liver proteins during ground squirrel hibernation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298(2):R329–40

Feketa VV, Nikolaev YA, Merriman DK, Bagriantsev SN, Gracheva EO. 2020. CNGA3 acts as a cold sensor in hypothalamic neurons. *eLife* 9:e55370

Feng NY, Junkins MS, Merriman DK, Bagriantsev SN, Gracheva EO. 2019. Osmolyte depletion and thirst suppression allow hibernators to survive for months without water. *Curr. Biol.* 29(18):3053–58.e3

Florant GL, Healy JE. 2012. The regulation of food intake in mammalian hibernators: a review. *J. Comp. Physiol. B* 182(4):451–67

Florant GL, Hester L, Ameenuddin S, Rintoul DA. 1993. The effect of a low essential fatty acid diet on hibernation in marmots. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 264(4):R747–53

Florant GL, Lawrence AK, Williams K, Bauman WA. 1985. Seasonal changes in pancreatic B-cell function in euthermic yellow-bellied marmots. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 249(2):R159–65

Florant GL, Porst H, Peiffer A, Hudachek SF, Pittman C, et al. 2004. Fat-cell mass, serum leptin and adiponectin changes during weight gain and loss in yellow-bellied marmots (*Marmota flaviventris*). *J. Comp. Physiol. B* 174(8):633–39

Florant GL, Singer L, Scheurink AJW, Park CR, Richardson RD, Woods SC. 1991. Intraventricular insulin reduces food intake and body weight of marmots during the summer feeding period. *Physiol. Behav.* 49(2):335–38

Folk GE. 1974. *Textbook of Environmental Physiology*. Philadelphia: Lea & Febiger

Frank CL. 1994. Polyunsaturate content and diet selection by ground squirrels (*Spermophilus lateralis*). *Ecology* 75(2):458–63

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. *Nature* 395(6704):763–70

Gähwiler BH, Mamoon AM, Schlapfer WT, Tobias CA. 1972. Effects of temperature on spontaneous bioelectric activity of cultured nerve cells. *Brain Res.* 40(2):527–33

Galster W, Morrison P. 1975. Gluconeogenesis in Arctic ground squirrels between periods of hibernation. *Am. J. Physiol.* 228(1):325–30

Gao Q, Horvath TL. 2007. Neurobiology of feeding and energy expenditure. *Annu. Rev. Neurosci.* 30:367–98

Gao Y-F, Wang J, Wang H-P, Feng B, Dang K, et al. 2012. Skeletal muscle is protected from disuse in hibernating Daurian ground squirrels. *Comp. Biochem. Physiol. A* 161(3):296–300

Gehnrich SC, Aprille JR. 1988. Hepatic gluconeogenesis and mitochondrial function during hibernation. *Comp. Biochem. Physiol. B* 91(1):11–16

Geiser F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* 66:239–74

Geiser F. 2008. Ontogeny and phylogeny of endothermy and torpor in mammals and birds. *Comp. Biochem. Physiol. A* 150(2):176–80

Geiser F, Kenagy GJ. 1987. Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 252(5):R897–901

Geiser F, Kenagy GJ. 1988. Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. *Physiol. Zool.* 61(5):442–49

Geiser F, Ruf T. 1995. Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol. Zool.* 68(6):935–66

Gessner C. 1551. Lib. I. de Quadrupedibus viviparis. In *Conradi Gesneri medici Tigurini Historiae Animalium*, pp. 842–1096. **https://www.biodiversitylibrary.org/item/136711#page/886/mode/1up**

Goldman SS, Willis JS. 1973. Cold resistance of the brain during hibernation: II. Na-K-activated ATPase. *Cryobiology* 10(3):218–24

Grabek KR, Cooke TF, Epperson LE, Spees KK, Cabral GF, et al. 2019. Genetic variation drives seasonal onset of hibernation in the 13-lined ground squirrel. *Commun. Biol.* 2:478

Grabek KR, Martin SL, Hindle AG. 2015. Proteomics approaches shed new light on hibernation physiology. *J. Comp. Physiol. B* 185(6):607–27

Green CJ, Brosnan JT, Fuller BJ, Lowry M, Stubbs M, Ross BD. 1984. Effect of hibernation on liver and kidney metabolism in 13-lined ground squirrels. *Comp. Biochem. Physiol. B* 79(2):167–71

Grigg GC, Beard LA, Augee ML. 2004. The evolution of endothermy and its diversity in mammals and birds. *Physiol. Biochem. Zool.* 77(6):982–97

Guo Q, Mi X, Sun X, Li X, Fu W, et al. 2017. Remarkable plasticity of Na^+, K^+-ATPase, Ca^{2+}-ATPase and SERCA contributes to muscle disuse atrophy resistance in hibernating Daurian ground squirrels. *Sci. Rep.* 7(1):10509

Gwinner E. 1986. *Circannual Rhythms*. Heidelberg, Ger.: Springer

Hahn TM, Breininger JF, Baskin DG, Schwartz MW. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat. Neurosci.* 1(4):271–72

Hamilton JD, Pfeiffer EW. 1977. Effects of cold exposure and dehydration on renal function in black-tailed prairie dogs. *J. Appl. Physiol.* 42(2):295–99

Harlow HJ. 1997. Winter body fat, food consumption and nonshivering thermogenesis of representative spontaneous and facultative hibernators: the white-tailed prairie dog and black-tailed prairie dog. *J. Therm. Biol.* 22(1):21–30

Hazlerigg DG, Lincoln GA. 2011. Hypothesis: Cyclical histogenesis is the basis of circannual timing. *J. Biol. Rhythm.* 26(6):471–85

Healy JE, Bateman JL, Ostrom CE, Florant GL. 2011. Peripheral ghrelin stimulates feeding behavior and positive energy balance in a sciurid hibernator. *Horm. Behav.* 59(4):512–19

Healy JE, Ostrom CE, Wilkerson GK, Florant GL. 2010. Plasma ghrelin concentrations change with physiological state in a sciurid hibernator (*Spermophilus lateralis*). *Gen. Comp. Endocrinol.* 166(2):372–78

Heldmaier G, Ortmann S, Elvert R. 2004. Natural hypometabolism during hibernation and daily torpor in mammals. *Respir. Physiol. Neurobiol.* 141(3):317–29

Heller HC. 1979. Hibernation: neural aspects. *Annu. Rev. Physiol.* 41:305–21

Heller HC, Colliver GW, Beard J. 1977. Thermoregulation during entrance into hibernation. *Pflüg. Arch.* 369(1):55–59

Heller HC, Ruby NF. 2004. Sleep and circadian rhythms in mammalian torpor. *Annu. Rev. Physiol.* 66:275–89

Hendriks KDW, Joschko CP, Hoogstra-Berends F, Heegsma J, Faber K-N, Henning RH. 2020. Hibernator-derived cells show superior protection and survival in hypothermia compared to non-hibernator cells. *Int. J. Mol. Sci.* 21(5):1864

Hindle AG, Karimpour-Fard A, Epperson LE, Hunter LE, Martin SL. 2011. Skeletal muscle proteomics: Carbohydrate metabolism oscillates with seasonal and torpor-arousal physiology of hibernation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301(5):R1440–52

Hoffstaetter LJ, Mastrotto M, Merriman DK, Dib-Hajj SD, Waxman SG, et al. 2018. Somatosensory neurons enter a state of altered excitability during hibernation. *Curr. Biol.* 28(18):2998–3004.e3

Horwitz BA, Chau SM, Hamilton JS, Song C, Gorgone J, et al. 2013. Temporal relationships of blood pressure, heart rate, baroreflex function, and body temperature change over a hibernation bout in Syrian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305(7):R759–68

Huang X, Koutcherov I, Lin S, Wang H, Storlien L. 1996. Localization of leptin receptor mRNA expression in mouse brain. *Neuroreport* 7(15–17):2635–38

Jani A, Martin SL, Jain S, Keys D, Edelstein CL. 2013. Renal adaptation during hibernation. *Am. J. Physiol. Ren. Physiol.* 305(11):F1521–32

Jethwa PH, Warner A, Nilaweera KN, Brameld JM, Keyte JW, et al. 2007. VGF-derived peptide, TLQP-21, regulates food intake and body weight in Siberian hamsters. *Endocrinology* 148(8):4044–55
Julius D. 2013. TRP channels and pain. *Annu. Rev. Cell Dev. Biol.* 29:355–84
Kahn SE, Hull RL, Utzschneider KM. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444(7121):840–46
Kehl TH, Morrison P. 1960. Peripheral nerve function and hibernation in the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Bull. Mus. Comp. Zool. Harv.* 124:387–403
Kenagy GJ. 1980. Interrelation of endogenous annual rhythms of reproduction and hibernation in the golden-mantled ground squirrel. *J. Comp. Physiol.* 135(4):333–39
Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402(6762):656–60
Kolomiytseva IK, Perepelkina NI, Zharikova AD, Popov VI. 2008. Membrane lipids and morphology of brain cortex synaptosomes isolated from hibernating Yakutian ground squirrel. *Comp. Biochem. Physiol. B* 151(4):386–91
Krilowicz BL, Edgar DM, Heller HC. 1989. Action potential duration increases as body temperature decreases during hibernation. *Brain Res.* 498(1):73–80
Krystal AD, Schopler B, Kobbe S, Williams C, Rakatondrainibe H, et al. 2013. The relationship of sleep with temperature and metabolic rate in a hibernating primate. *PLOS ONE* 8(9):e69914
Labrecque N, Cermakian N. 2015. Circadian clocks in the immune system. *J. Biol. Rhythm.* 30(4):277–90
Langlet F, Levin BE, Luquet S, Mazzone M, Messina A, et al. 2013. Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. *Cell Metab.* 17(4):607–17
Larkin JE, Heller HC. 1999. Sleep after arousal from hibernation is not homeostatically regulated. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 276(2):R522–29
Laursen WJ, Mastrotto M, Pesta D, Funk OH, Goodman JB, et al. 2015. Neuronal UCP1 expression suggests a mechanism for local thermogenesis during hibernation. *PNAS* 112(5):1607–12
Laursen WJ, Schneider ER, Merriman DK, Bagriantsev SN, Gracheva EO. 2016. Low-cost functional plasticity of TRPV1 supports heat tolerance in squirrels and camels. *PNAS* 113(40):11342–47
Lee TM, Zucker I. 1991. Suprachiasmatic nucleus and photic entrainment of circannual rhythms in ground squirrels. *J. Biol. Rhythm.* 9(4):315–30
Leib DE, Zimmerman CA, Knight ZA. 2016. Thirst. *Curr. Biol.* 26(24):R1260–65
León-Espinosa G, Antón-Fernández A, Tapia-González S, DeFelipe J, Muñoz A. 2018. Modifications of the axon initial segment during the hibernation of the Syrian hamster. *Brain Struct. Funct.* 223(9):4307–21
Lewis JE, Ebling FJP. 2017. Tanycytes as regulators of seasonal cycles in neuroendocrine function. *Front. Neurol.* 8:79
Lovegrove BG. 2012. A single origin of heterothermy in mammals. In *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*, ed. T Ruf, C Bieber, W Arnold, E Millesi, pp. 3–11. Berlin: Springer
Lusk G. 1924. Animal calorimetry: twenty-fourth paper. Analysis of the oxidation of mixtures of carbohydrate and fat. *J. Biol. Chem.* 59(1):41–42
Lyman CP, O'Brien RC. 1963. Autonomic control of circulation during the hibernating cycle in ground squirrels. *J. Physiol.* 168(3):477–99
Lyman CP, Willis JS, Malan A, Wang LCH. 1982. *Hibernation and Torpor in Mammals and Birds*. New York: Academic
MacDonald JA, Storey KB. 1999. Regulation of ground squirrel Na^+K^+-ATPase activity by reversible phosphorylation during hibernation. *Biochem. Biophys. Res. Commun.* 254(2):424–29
Malan A. 2010. Is the torpor-arousal cycle of hibernation controlled by a non-temperature-compensated circadian clock? *J. Biol. Rhythm.* 25(3):166–75
Mandelblat-Cerf Y, Kim A, Burgess CR, Subramanian S, Tannous BA, et al. 2017. Bidirectional anticipation of future osmotic challenges by vasopressin neurons. *Neuron* 93(1):57–65
Marcheva B, Ramsey KM, Peek CB, Affinati A, Maury E, Bass J. 2013. Circadian clocks and metabolism. *Handb. Exp. Pharmacol.* 217:127–55
Masento NA, Golightly M, Field DT, Butler LT, van Reekum CM. 2014. Effects of hydration status on cognitive performance and mood. *Br. J. Nutr.* 111(10):1841–52

Mateo JM, Johnston RE. 2000. Retention of social recognition after hibernation in Belding's ground squirrels. *Anim. Behav.* 59(3):491–99

Matos-Cruz V, Schneider ER, Mastrotto M, Merriman DK, Bagriantsev SN, Gracheva EO. 2017. Molecular prerequisites for diminished cold sensitivity in ground squirrels and hamsters. *Cell Rep.* 21(12):3329–37

Maury E. 2019. Off the clock: from circadian disruption to metabolic disease. *Int. J. Mol. Sci.* 20(7):1597

McKemy DD, Neuhausser WM, Julius D. 2002. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416(6876):52–58

Millesi E, Prossinger H, Dittami JP, Fieder M. 2001. Hibernation effects on memory in European ground squirrels (*Spermophilus citellus*). *J. Biol. Rhythm.* 16(3):264–71

Mrosovsky N. 1975. The amplitude and period of circannual cycles of body weight in golden-mantled ground squirrels with medial hypothalamic lesions. *Brain Res.* 99(1):97–116

Mrosovsky N, Boshes M. 1986. Meal patterns and food intakes of ground squirrels during circannual cycles. *Appetite* 7(2):163–75

Mugahid DA, Sengul TG, You X, Wang Y, Steil L, et al. 2019. Proteomic and transcriptomic changes in hibernating grizzly bears reveal metabolic and signaling pathways that protect against muscle atrophy. *Sci. Rep.* 9:19975

Muleme HM, Walpole AC, Staples JF. 2006. Mitochondrial metabolism in hibernation: metabolic suppression, temperature effects, and substrate preferences. *Physiol. Biochem. Zool.* 79(3):474–83

Niswender KD, Schwartz MW. 2003. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front. Neuroendocrinol.* 24(1):1–10

Ollmann MM, Wilson BD, Yang Y-K, Kerns JA, Chen Y, et al. 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278(5335):135–38

Olson JM, Jinka TR, Larson LK, Danielson JJ, Moore JT, et al. 2013. Circannual rhythm in body temperature, torpor, and sensitivity to A_1 adenosine receptor agonist in Arctic ground squirrels. *J. Biol. Rhythm.* 28(3):201–7

Ormseth OA, Nicolson M, Pelleymounter MA, Boyer BB. 1996. Leptin inhibits prehibernation hyperphagia and reduces body weight in Arctic ground squirrels. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 271(6):R1775–79

Ou J, Ball JM, Luan Y, Zhao T, Miyagishima KJ, et al. 2018. iPSCs from a hibernator provide a platform for studying cold adaptation and its potential medical applications. *Cell* 173(4):851–63.e16

Oxf. Univ. Press. 2020. Hibernation. *Oxford English Dictionary*. **https://www.oed.com/view/Entry/86665?rskey=0xRn2r&result=1&isAdvanced=false#eid**

Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, et al. 2002. A heat-sensitive TRP channel expressed in keratinocytes. *Science* 296(5575):2046–49

Pengelley ET, Asmundson SJ, Barnes B, Aloia RC. 1976. Relationship of light intensity and photoperiod to circannual rhythmicity in the hibernating ground squirrel, *Citellus lateralis*. *Comp. Biochem. Physiol. A Physiol.* 53(3):273–77

Pengelley ET, Fisher KC. 1963. The effect of temperature and photoperiod on the yearly hibernating behavior of captive golden-mantled ground squirrels (*Citellus lateralis tescorum*). *Can. J. Zool.* 41(6):1103–20

Pliny. 77 CE. *The Natural History of Pliny*, transl. J Bostock, HT Riley, Vol. 2. 311. Project Gutenberg. **http://www.gutenberg.org/files/57493/57493-h/57493-h.htm**

Popov VI, Medvedev NI, Patrushev IV, Ignat'ev DA, Morenkov ED, Stewart MG. 2007. Reversible reduction in dendritic spines in CA1 of rat and ground squirrel subjected to hypothermia-normothermia in vivo: a three-dimensional electron microscope study. *Neuroscience* 149(3):549–60

Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, et al. 2004. Adiponectin acts in the brain to decrease body weight. *Nat. Med.* 10(5):524–29

Reddy AB, Rey G. 2014. Metabolic and nontranscriptional circadian clocks: eukaryotes. *Annu. Rev. Biochem.* 83:165–89

Regan MD, Chiang E, Martin SL, Porter WP, Assadi-Porter FM, Carey HV. 2019. Shifts in metabolic fuel use coincide with maximal rates of ventilation and body surface rewarming in an arousing hibernator. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 316(6):R764–75

Roth TC, Rattenborg NC, Pravosudov VV. 2010. The ecological relevance of sleep: the trade-off between sleep, memory and energy conservation. *Philos. Trans. R. Soc. B* 365(1542):945–59

Ruby NF, Dark J, Heller HC, Zucker I. 1998. Suprachiasmatic nucleus: role in circannual body mass and hibernation rhythms of ground squirrels. *Brain Res.* 782(1):63–72

Ruczynski I, Siemers BM. 2011. Hibernation does not affect memory retention in bats. *Biol. Lett.* 7(1):153–55

Ruediger J, Van der Zee EA, Strijkstra AM, Aschoff A, Daan S, Hut RA. 2007. Dynamics in the ultrastructure of asymmetric axospinous synapses in the frontal cortex of hibernating European ground squirrels (*Spermophilus citellus*). *Synapse* 61(5):343–52

Ruf T, Arnold W. 2008. Effects of polyunsaturated fatty acids on hibernation and torpor: a review and hypothesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294(3):R1044–52

Ruf T, Geiser F. 2015. Daily torpor and hibernation in birds and mammals. *Biol. Rev.* 90(3):891–926

Russ U, Siemen D. 1996. Kinetic parameters of the ionic currents in myelinated axons: characterization of temperature effects in a hibernator and a nonhibernator. *Pflüg. Arch.* 431(6):888–94

Sáenz de Miera C, Monecke S, Bartzen-Sprauer J, Laran-Chich M-P, Pévet P, et al. 2014. A circannual clock drives expression of genes central for seasonal reproduction. *Curr. Biol.* 24(13):1500–6

Schwartz C, Andrews MT. 2013. Circannual transitions in gene expression: lessons from seasonal adaptations. *Curr. Top. Dev. Biol.* 105:247–73

Schwartz C, Ballinger MA, Andrews MT. 2015a. Melatonin receptor signaling contributes to neuroprotection upon arousal from torpor in thirteen-lined ground squirrels. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 309(10):R1292–300

Schwartz C, Hampton M, Andrews MT. 2013. Seasonal and regional differences in gene expression in the brain of a hibernating mammal. *PLOS ONE* 8(3):e58427

Schwartz C, Hampton M, Andrews MT. 2015b. Hypothalamic gene expression underlying pre-hibernation satiety. *Genes Brain Behav.* 14(3):310–18

Sharma S, Kavuru M. 2010. Sleep and metabolism: an overview. *Int. J. Endocrinol.* 2010:270832

Sheriff MJ, Fridinger RW, Tøien Ø, Barnes BM, Buck CL. 2013. Metabolic rate and prehibernation fattening in free-living Arctic ground squirrels. *Physiol. Biochem. Zool.* 86(5):515–27

South FE. 1961. Phrenic nerve-diaphragm preparations in relation to temperature and hibernation. *Am. J. Physiol.* 200(3):565–71

Spector DA, Deng J, Coleman R, Wade JB. 2015. The urothelium of a hibernator: the American black bear. *Physiol. Rep.* 3(6):e12429

Squire TL, Lowe ME, Bauer VW, Andrews MT. 2003. Pancreatic triacylglycerol lipase in a hibernating mammal. II. Cold-adapted function and differential expression. *Physiol. Genom.* 16(1):131–40

Stanley BG, Leibowitz SF. 1984. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci.* 35(26):2635–42

Staples JF. 2014. Metabolic suppression in mammalian hibernation: the role of mitochondria. *J. Exp. Biol.* 217(12):2032–36

Staples JF. 2016. Metabolic flexibility: hibernation, torpor, and estivation. *Compr. Physiol.* 6(2):737–71

Staples JF, Brown JCL. 2008. Mitochondrial metabolism in hibernation and daily torpor: a review. *J. Comp. Physiol. B* 178(7):811–27

Steward C, Horan T, Schuhler S, Bennett G, Ebling F. 2003. Central administration of thyrotropin releasing hormone (TRH) and related peptides inhibits feeding behavior in the Siberian hamster. *Neuroreport* 14(5):687–91

Storey KB, Storey JM. 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Q. Rev. Biol.* 65(2):145–74

Strijkstra AM, Daan S. 1998. Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 275(4):R1110–17

Strijkstra AM, Hut RA, de Wilde MC, Stieler J, Van der Zee EA. 2003. Hippocampal synaptophysin immunoreactivity is reduced during natural hypothermia in ground squirrels. *Neurosci. Lett.* 344(1):29–32

Strumwasser F. 1958. Thermoregulatory, brain and behavioral mechanisms during entrance into hibernation in the squirrel, *Citellus beecheyi*. *Am. J. Physiol.* 196(1):15–22

Suri LNM, Cruz A, Veldhuizen RAW, Staples JF, Possmayer F, et al. 2013. Adaptations to hibernation in lung surfactant composition of 13-lined ground squirrels influence surfactant lipid phase segregation properties. *Biochim. Biophys. Acta Biomembr.* 1828(8):1707–14

Suri LNM, McCaig L, Picardi MV, Ospina OL, Veldhuizen RAW, et al. 2012. Adaptation to low body temperature influences pulmonary surfactant composition thereby increasing fluidity while maintaining appropriately ordered membrane structure and surface activity. *Biochim. Biophys. Acta Biomembr.* 1818(7):1581–89

Tøien Ø, Blake J, Edgar DM, Grahn DA, Heller HC, Barnes BM. 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* 331(6019):906–9

Turbill C, Bieber C, Ruf T. 2011. Hibernation is associated with increased survival and the evolution of slow life histories among mammals. *Proc. R. Soc. B* 278(1723):3355–63

van Breukelen F, Martin SL. 2015. The hibernation continuum: physiological and molecular aspects of metabolic plasticity in mammals. *Physiology* 30(4):273–81

von der Ohe CG, Darian-Smith C, Garner CC, Heller HC. 2006. Ubiquitous and temperature-dependent neural plasticity in hibernators. *J. Neurosci.* 26(41):10590–98

von der Ohe CG, Garner CC, Darian-Smith C, Heller HC. 2007. Synaptic protein dynamics in hibernation. *J. Neurosci.* 27(1):84–92

Walker JM, Glotzbach SF, Berger RJ, Heller HC. 1977. Sleep and hibernation in ground squirrels (*Citellus* spp.): electrophysiological observations. *Am. J. Physiol.* 233(5):R213–21

Wang LCH. 1979. Time patterns and metabolic rates of natural torpor in the Richardson's ground squirrel. *Can. J. Zool.* 57(1):149–55

Waterson MJ, Horvath TL. 2015. Neuronal regulation of energy homeostasis: beyond the hypothalamus and feeding. *Cell Metab.* 22(6):962–70

Wikelski M, Martin LB, Scheuerlein A, Robinson MT, Robinson ND, et al. 2008. Avian circannual clocks: adaptive significance and possible involvement of energy turnover in their proximate control. *Philos. Trans. R. Soc. B* 363(1490):411–23

Williams CT, Barnes BM, Kenagy GJ, Buck CL. 2014. Phenology of hibernation and reproduction in ground squirrels: integration of environmental cues with endogenous programming. *J. Zool.* 292(2):112–24

Wu C-W, Biggar KK, Storey KB. 2013. Biochemical adaptations of mammalian hibernation: exploring squirrels as a perspective model for naturally induced reversible insulin resistance. *Braz. J. Med. Biol. Res.* 46(1):1–13

Xing X, Tang G-B, Sun M-Y, Yu C, Song S-Y, et al. 2016. Leptin regulates energy intake but fails to facilitate hibernation in fattening Daurian ground squirrels (*Spermophilus dauricus*). *J. Therm. Biol.* 57:35–43

Xing X, Yang M, Wang D-H. 2015. The expression of leptin, hypothalamic neuropeptides and UCP1 before, during and after fattening in the Daurian ground squirrel (*Spermophilus dauricus*). *Comp. Biochem. Physiol. A* 184:105–12

Zimmerman CA, Leib DE, Knight ZA. 2017. Neural circuits underlying thirst and fluid homeostasis. *Nat. Rev. Neurosci.* 18(8):459–69

Zucker I, Boshes M, Dark J. 1983. Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 244(4):R472–80