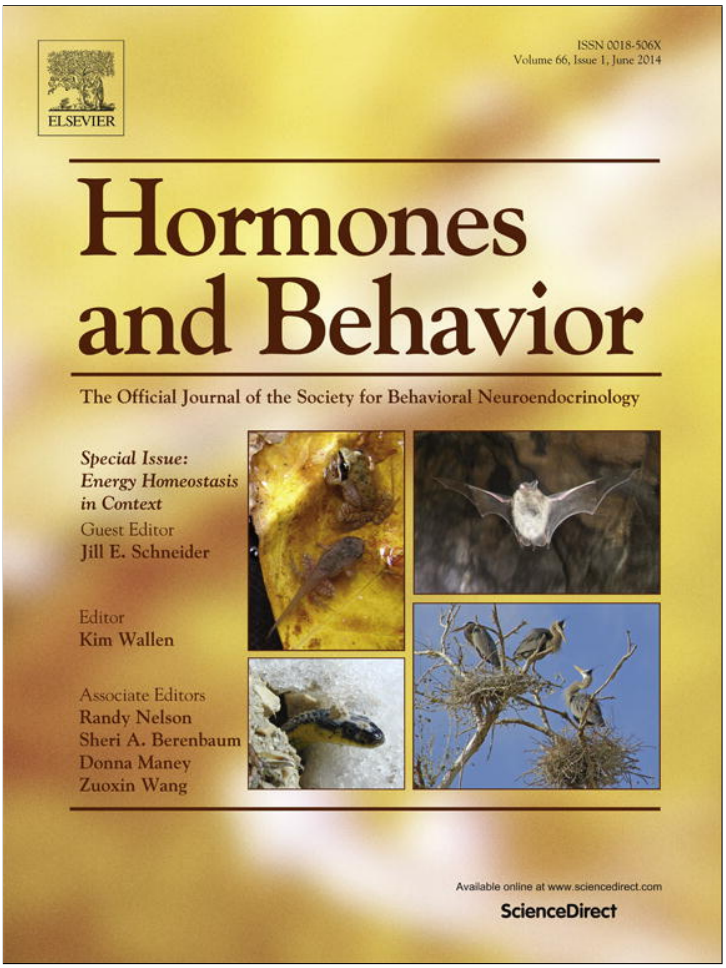

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Hormones and hibernation: possible links between hormone systems, winter energy balance and white-nose syndrome in bats

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ABSTRACT

This article is part of a Special Issue "Energy Balance".

Hibernation allows mammals to survive in cold climates and during times of reduced food availability. Drastic physiological changes are required to maintain the energy savings that characterize hibernation. These changes presumably enable adjustments in endocrine activity that control metabolism and body temperature, and ultimately influence expression of torpor and periodic arousals. Despite challenges that exist when examining hormonal pathways in small-bodied hibernators, bats represent a potential model taxon for comparative neuro-endocrinological studies of hibernation due to their diversity of species and the reliance of many species on heterothermy. Understanding physiological mechanisms underlying hibernation in bats is also important from a conservation physiology perspective due to white-nose syndrome, an emerging infectious disease causing catastrophic mortality among hibernating bats in eastern North America. Here we review the potential influence of three key hormonal mechanisms – leptin, melatonin and glucocorticoids – on hibernation in mammals with an emphasis on bats. We propose testable hypotheses about potential effects of WNS on these systems and their evolution.

Contents

Introduction

Mammalian hibernation is characterized by bouts of torpor, or periods of reduced body temperature (T_b) and metabolic rate (MR) that can last from days to weeks, interspersed with short periods of activity and normothermia (Geiser, 2004, 2013). Torpor is primarily considered a response to energy imbalance associated with low ambient temperatures (T_a) and/or reduced food supply (Geiser, 2004, 2013), although a number of other benefits of torpor have been identified (Geiser and Brigham, 2012). In hibernators T_b during torpor can be reduced well below 10 °C and MR can fall to less than 1% of that for normothermic individuals at the same T_a (Geiser, 2013). A wide range of physiological functions are down-regulated during torpor. Heart rate slows dramatically (e.g., less than 5 beats/min in the thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*), Johnson, 1929), respiratory rate declines (e.g., less than 2 breaths/min in *I. tridecemlineatus*, Johnson, 1929), and digestive/excretory functions are inhibited (Zatzman, 1984). However, at some interval (i.e., days to weeks) all mammalian hibernators periodically rewarm to a normothermic T_b for short periods, during which autonomic functions are rapidly restored to re-establish homeostasis (Geiser, 2004, 2013). Rewarming depends primarily on the metabolism of brown adipose

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tissue (BAT) first warming the brain and thoracic cavity, leading eventually to normothermia (Geiser, 2004; Ootsuka et al., 2009; Smith and Hock, 1963). Arousals are energetically expensive and account for most of the over-winter energy budget (Geiser, 2004; Wang, 1978).

The dramatic seasonal changes in behavior and energy expenditure of hibernators, and the repeated short-term adjustments in physiological processes associated with periodic arousals, reflect a range of neuroendocrine mechanisms acting on a range of behavioral and physiological traits from feeding behavior in the fall to thermoregulation and metabolism throughout winter (Nürnberger, 1995). In general, many endocrine functions are thought to be depressed at the beginning of hibernation, resume after mid-hibernation, and peak after spring emergence (Wang, 1982), although responses depend on the target structure and the expression of hormone receptors (Srivastava and Krishna, 2010). The timing of entry into and emergence from hibernation are tightly linked to reproductive phenology (Jonasson and Willis, 2011; Michener, 1979, 1992), suggesting seasonal fluctuations in some of the same hormone-receptor systems may be important for both hibernation and reproduction. For example, leptin sensitivity is adjusted during both pre-hibernation hyperphagia and post-hibernation reproduction (Kronfeld-Schor et al., 2000; Kunz et al., 1999; Kwiecinski and Damassa, 2000; Widmaier et al., 1997). Melatonin appears to help entrain seasonal rhythms in reproduction and thermoregulation (Barrenetxe et al., 2004). Periodic arousals appear to be correlated with hormonal stress responses (Kwiecinski and Damassa, 2000; Popova and Koryakina, 1981), and stress hormones can affect both fat deposition (Dallman et al., 2000) and reproductive function (Rivest and Rivier, 1995; Whirledge and Cidlowski, 2010). Although links between hormone secretion during hibernation and reproduction are not fully understood, similarities suggest that similar mechanisms may influence energetics of both aspects of the annual cycle for hibernators.

Identifying hormonal mechanisms associated with hibernation is challenging but some model taxa have potential to help improve our understanding. Neuroendocrine regulation of hibernation in temperate-zone bats has received relatively little attention in part because bats are small, providing only small blood samples for analysis, and because many species can be difficult to keep and breed in captivity. Nonetheless, several characteristics of temperate-zone, insectivorous bats recommend them for endocrine studies of hibernation. In contrast to rodent hibernators, most of which spend the winter alone in enclosed burrows, bats aggregate in large numbers and often use open-air hibernacula from which they can be readily captured. This enables relatively large sample sizes of naturally hibernating animals, although obviously care must be taken to minimize impacts of research on wild populations. Methods have recently been improved to allow even bat species that are challenging to maintain in captivity to hibernate naturally in the laboratory (e.g., Barnard, 2009; Brownlee-Bouboulis and Reeder, 2013; Lorch et al., 2011; Warnecke et al., 2012). Insect-eating bats are also extremely diverse and highly prone to heterothermy. At least hundreds of species likely express some degree of heterothermy and dozens likely exhibit classic hibernation (Stawski et al., 2013) creating great potential for studies of comparative endocrinology of hibernation. Hibernating bats also exhibit an atypical pattern of reproduction compared to many hibernators. Mating occurs in the fall and early winter prior to hibernation and females then store sperm or delay implantation until spring and become pregnant if they are in good body condition (Racey and Entwistle, 2000). This provides an opportunity to study connections between the timing of hormonal influence on reproduction and hibernation by comparison to rodent hibernators that mate and rear their young in the spring.

Understanding hormonal regulation of bat hibernation has also recently become important from a conservation physiology perspective (Cooke et al., 2013). White-nose syndrome (WNS) is an emerging infectious disease of hibernating bats caused by the cold-tolerant fungus *Pseudogymnoascus destructans* (*Pd;* formerly *Geomyces destructans*, Minnis and Lindner, 2013). *Pd* has been found in hibernacula, and/or on bats, of 11 species in eastern North America with clinical signs of WNS in seven species (U.S. Fish and Wildlife Service, 2014). Since its first occurrence in New York State in 2007, WNS has spread to 22 U.S. states and five Canadian provinces, with millions of bats killed and mortality rates as high as 99% in some hibernacula (Frick et al., 2010a; U.S. Fish and Wildlife Service, 2012). The disease is clinically defined based on the pathology of infection of exposed skin with *Pd* (Gargas et al., 2009; Lorch et al., 2011; Meteyer et al., 2009) but mortality is ultimately caused by increased arousal frequency and premature depletion of fat stores during hibernation (Boyles and Willis, 2010; Reeder etal., 2012; Warnecke et al., 2012). Loss of fluid across lesions caused by the fungus is thought to result in hypotonic dehydration, hypovolemia and metabolic acidosis (Cryan et al., 2010, 2013; Warnecke et al., 2013; Willis et al., 2011), and Warnecke et al. (2013) proposed a conceptual model connecting these clinical signs with mortality.

Despite high mortality, some individuals appear to survive and recover as evidenced from scars on the wings the summer following infection (Fuller et al., 2011; Reichard and Kunz, 2009), but phenotypic or genetic differences of survivors have yet to be identified. Comparison of neuroendocrine function in infected and uninfected bats, targeting specific hormonal pathways at multiple time points throughout the annual cycle, has potential to help test specific hypotheses about the pathophysiological and reproductive consequences of WNS. These studies could help us understand why bats with WNS arouse too often during hibernation, predict potential reproductive consequences of WNS, and aid in development of management strategies to help more bats survive and, importantly, reproduce.

Here we review the literature on several key hormonal signals implicated in mammalian hibernation with a focus on bats where data are available. The phenology of hibernation and reproduction are tightly linked in hibernators. Therefore, although there will be many signals influencing hibernation behavior and physiology, we focus on three that have implications for both overwinter and reproductive energy

Table 1
Summary of three key hormone systems – leptin, melatonin and glucocorticoids (GCs) – implicated in both mammalian hibernation and reproduction.

System	Role in hibernation	Role in reproduction	references
Leptin	- Controls energy intake by regulating food consumption and metabolism - Regulates uncoupling proteins therefore influencing non-shivering thermogenesis	- May regulate energy budgets during pregnancy	Gong et al., 1997; Kronfeld-Schor et al., 2000; Scarpace et al., 1997; Trujillo et al., 2011
Melatonin	- Influences the circadian and circannual rhythms for thermoregulation and metabolism - Influences glucose homeostasis - Influences non-shivering thermogenesis	- Influences seasonality of mating behavior and physiology. - Affects the release of gonadotropins	Banerjee et al., 2009; Beasley et al., 1984; Brown, 1984; Darrow et al., 1986; Heldmaier et al., 1981; Illnerova, 1991; Reiter et al., 1974; Tamarkin et al., 1976; Wang, 1982
GCs	- Increases energy availability in circulation for pre-hibernation fattening and hibernation metabolism - Mediates physiological stress response (e.g., stress reactivity during periodic arousals) - Influences immune function and inflammatory response	- Elevated GCs suppress reproductive function - Can inhibit release of gonadotropin-releasing hormone - May reduce expression of luteinizing hormone	Baldwin, 1979; O'Connor et al., 2000; Reichardt et al., 2001; Rivest and Rivier, 1995; Whirledge and Cidlowski, 2010

Table 2
Pathophysiological and potential selective effects of white nose syndrome (WNS) on leptin, melatonin and glucocorticoid (GC) hormone systems. IRIS refers to immune reconstitution inflammatory syndrome (Meteyer et al., 2012, see text for explanation).

System	Hypothesis	Predictions
Leptin	- Fall leptin sensitivity influences fat accumulation and the overwinter energy budget.	- Bats with lowest leptin sensitivity in fall will accumulate larger fat reserves and exhibit higher survival. - Leptin sensitivity will be lower in post-WNS populations.
Melatonin	- Stronger photoperiod cues near hibernaculum entrances influence arousal behavior. - Increased arousal frequency augments melatonin levels. - Fall melatonin levels influence fat accumulation and the winter energy budget.	- Bats near cave entrances will exhibit greater diurnal fluctuations in melatonin. - Greater diurnal melatonin fluctuations will increase the chance of bats emerging at night when food might be available. - After controlling for photoperiod, melatonin levels will increase as *Pd* infection progresses. - Fall melatonin levels will be higher in survivors and in post-WNS populations because of enhanced potential for fat accumulation.
GCs	- Repeated arousals by infected bats represent a stressor for other bats in the hibernaculum which increases their arousal frequency beyond infection alone - Baseline GCs in spring affect the severity of IRIS during healing and recovery. - Sustained elevation of GCs in spring will affect reproduction	- GC levels will be highest for bats in infected hibernacula with large populations (and many bats rewarming too frequently) - WNS will select for individuals with a smallest GC stress response in winter. - Bats with the highest levels of baseline glucocorticoids in spring will have a reduced likelihood of IRIS and enhanced survival. - Delayed parturition, reduced offspring survival and male-biased sex ratio will occur for affected populations.

balance and for which there are recent data from bats: leptin; melatonin; and glucocorticoids (Table 1). We highlight the potential of endocrinological studies of each of these systems to improve understanding of mechanisms underlying hibernation behavior and physiology of healthy bats, as well as mortality of bats from WNS. We also propose testable hypotheses about how WNS pathophysiology might influence the proximate function of these systems in the short term, and predict how the tremendous selective bottleneck currently being imposed on bat populations by WNS might influence adaptive evolution of these systems (Table 2).

Leptin

The protein hormone leptin influences metabolism and feeding behavior (Houseknecht and Spurlock, 2003) and it is well known that impaired leptin signaling can cause obesity in humans and mice (Myers et al., 2010; Sørensen et al., 1996). Given the importance of pre-hibernation fat accumulation and fat metabolism for many hibernators, regulation of leptin signaling is important for hibernation (e.g., Kronfeld-Schor et al., 2000; Srivastava and Krishna, 2008). In mammals, leptin is secreted by adipocytes in proportion with the size of the fat store, triggering satiety, increased MR, and increased behavioral activity (Cammisotto and Bendayan, 2007; Mistry et al., 1997). Ordinarily, high levels of circulating leptin lead to reduced food consumption so hibernators must mediate leptin signaling during the pre-hibernation period. High levels of circulating leptin could limit the quantity of fat stores gained during pre-hibernation and, in turn, affect overwinter energy budgets. In little brown bats (*Myotis lucifugus*) this problem is solved by a dissociation of leptin from adiposity, in combination with leptin resistance (Kronfeld-Schor et al., 2000). Levels of circulating leptin increase, as normal, with fat accumulation early during the pre-hibernation phase, but these levels then decline before the highest levels of adiposity are achieved (Kronfeld-Schor et al., 2000). Presumably disconnecting leptin from fat accumulation allows hibernators to continue to fatten despite substantial food consumption that would normally trigger satiety.

Leptin may also influence energy expenditure during hibernation via effects on lipolysis of white adipose tissue (WAT) during torpor (Yuan et al., 2011), and on non-shivering thermogenesis (NST) and BAT metabolism during arousals. In little brown bats, the capacity for NST rises during pre-hibernation, as fat mass and BAT mass increase (Kronfeld-Schor et al., 2000). Although data are sparse for hibernators, in common shrews (*Sorex araneus*) leptin secretion by brown adipocytes is highest in mid-winter, during the period of lowest body mass (Nieminen and Hyvärinen, 2000). This suggests that leptin is not only involved in energy intake, but may also serve as a signal that promotes or inhibits NST depending on the size of the BAT store (Nieminen and Hyvärinen, 2000). Leptin may even influence BAT metabolism itself given that it increases expression of uncoupling proteins (e.g., UCP1, UCP3) in BAT of rats (Gong et al., 1997; Scarpace et al., 1997). Uncoupling proteins increase the potential for heat production by decoupling electron transport from ATP production in mitochondrial membranes of brown adipocytes. Periodic arousals during hibernation require significant and rapid heat production from BAT. If patterns observed in rats are similar for hibernators, this suggests a role for leptin in arousal physiology and energetics in the midst of hibernation. Consistent with this hypothesis, recent genomic analyses suggest that the lipolytic activity of leptin has experienced positive selection in bats that regularly use heterothermy and, therefore, require the ability to mobilize fat reserves quickly (Yuan et al., 2011).

In addition to its potential influence on pre-hibernation and hibernation energetics, leptin also has the potential to influence the restoration of body condition and normal feeding behavior at the end of hibernation. As hibernators emerge and resume feeding, leptin levels rise with increasing body mass (Concannon et al., 2001). However, many species appear to maintain a temporary state of leptin resistance even after hibernation (Grattan et al., 2007; Trujillo et al., 2011) which could serve to increase energy acquisition to support early pregnancy after emergence. However, there is also potential for leptin to mediate feeding behavior of bats during pregnancy and lactation. After female bats emerge from hibernation, leptin levels rise throughout pregnancy but subsequently decrease dramatically after parturition in both little brown bats and big brown bats (*Eptesicus fuscus*) (Kunz et al., 1999; Kwiecinski and Damassa, 2000; Widmaier et al., 1997). As gestation proceeds, late pregnancy could be a time of reduced foraging efficiency for bats because pre-natal offspring represent a significant proportion of body mass that will increase wing loading and reduce maneuverability (Norberg and Rayner, 1987). After wing loading drops at parturition, foraging efficiency should improve which is convenient as lactation is the time of highest energetic costs for bats (Kurta et al., 1989). A hormonal signal that tends to limit (although not eliminate) foraging during late pregnancy, but then encourages intense feeding during lactation could be adaptive for reproductive female bats.

Premature depletion of fat stores during hibernation is a key characteristic of WNS in bats (Boyles and Willis, 2010; Warnecke et al., 2012). This suggests that bats that accumulate the largest fat stores in autumn should have a survival advantage following infection with *Pd*. Given the role of leptin as a satiety signal, this suggests that bats with the least sensitivity to leptin could accumulate the most mass in the fall and be more likely to survive and reproduce the following spring compared to other individuals. Plasma leptin levels are heritable, at least in humans (Narkiewicz et al., 1999; Rotimi et al., 1997) and likely heritable in other mammals. Although levels of heritability are not yet known for any behavioral or physiological trait associated with hibernation in bats,

the potential for strong selection on leptin sensitivity in combination with potentially high heritability creates the possibility that WNS could lead to rapid evolution of the leptin response or leptin sensitivity in surviving populations of bats. Thus, we hypothesize that bats characterized by the lowest leptin levels in autumn will exhibit greater survival from WNS and relatively high reproductive rates in spring. We also predict that fall leptin sensitivity should be lower in post-WNS populations compared to populations that have not yet been affected.

Melatonin

The role of melatonin as a signal influencing seasonal and diurnal biological rhythms is well known (e.g., Barrenetxe et al., 2004; Reiter, 1993). In general, darkness causes increased secretion of melatonin from the pineal gland with a range of consequences for circadian and circannual rhythms via interactions with the suprachiasmatic nucleus of the hypothalamus and melatonin receptors on a range of tissues (Brown, 1984; Illnerova, 1991). These effects include entrainment of seasonal rhythms for body mass and reproduction, regulation of diurnal fluctuations in T_b, and regulation of sleep-wake cycles (Anthony, 2000; Brown, 1984; Wang, 1982). Although relatively little is known about pineal activity in hibernating bats, seasonal periodicity of thermoregulation has been better studied in rodent hibernators and seasonal fluctuations in melatonin seem to be associated with pronounced variation in behavior and physiology between winter and summer (e.g., Duncan and Goldman, 2005a,b).

In general, short winter photoperiods trigger increased thermogenic capacity presumably via effects of increased nocturnal duration of melatonin secretion on capacity for thermogenesis (Heldmaier et al., 1981). In Siberian hamsters (*Phodopus sungorus*), a non-hibernator which can employ daily torpor, melatonin implants and/or short-day photoperiods increased the capacity for non-shivering thermogenesis, improved cold resistance (Heldmaier et al., 1981), and led to increased lipolysis and reduced body mass (Youngstrom and Bartness, 1995). By contrast, in Syrian hamsters (*Mesocricetus auratus*) short days and increased melatonin stimulated fat storage, presumably in preparation for hibernation (Wade and Bartness, 1984). Thus, as for its well-known role in reproductive and diurnal cycles, photoperiod and melatonin appear to provide context-specific seasonal cues that affect energy balance in both hibernators and non-hibernators.

For hibernators, melatonin secretion may also vary in the short-term between torpor and periodic arousals, even in the absence of photoperiod cues. In female Turkish hamsters (*Mesocricetus brandti*), melatonin synthesis and secretion were absent during torpor but reached levels comparable to those observed in the active season within the first day of a periodic arousal (Darrow et al., 1986). A similar pattern was observed in golden-mantled ground squirrels (*Spermophilus lateralis*) where melatonin levels were lowest during deep hibernation but rapidly increased within the first hour of an arousal (Stanton et al., 2005). Therefore, despite decreased melatonin output during torpor, spontaneous arousals appear to trigger melatonin secretion.

In bats, less is known about the influence of photoperiod and melatonin expression on seasonality of thermoregulation and torpor expression. However, in general, patterns of thermoregulation may be less seasonal compared to many highly seasonal rodent hibernators, like ground squirrels or hamsters. For instance, many bat species appear capable of pronounced heterothermy, including multi-day torpor bouts, throughout the active season and seem to rely on summer torpor as a matter of routine (e.g., Geiser and Ruf, 1995; Willis et al., 2006). Although food restriction can elicit torpor in some rodent hibernators, at least one bat species relies more heavily on summer torpor when it has greater energy reserves, presumably because this helps reduce predation risk associated with foraging (Stawski and Geiser, 2010). On the other hand, like many rodent hibernators, bats exhibit pronounced seasonal fluctuations in fat stores that may be influenced by photoperiod and melatonin (e.g., Jonasson and Willis, 2011). Recent work on bats also suggests a possible direct role for melatonin in hibernation energy balance and, specifically glucose metabolism. Srivastava and Krishna (2010) found that melatonin increased in autumn and early winter during the period of most pronounced fattening in greater Asiatic yellow bats (*Scotophilus heathii*). They also showed that melatonin injected during both the fall fattening period and in spring, removed insulin resistance and improved clearance of glucose from the blood. Although cellular mechanisms were not suggested, presumably melatonin somehow favored intracellular uptake of glucose for allocation to storage as glycogen in muscle tissue, or storage as fat. These results should be interpreted cautiously as concentrations of melatonin in treatment injections (1 ug/g body mass) were very high against normal background melatonin levels (on the pg/ml scale) (Srivastava and Krishna, 2010). However, these data raise the possibility of a direct role for melatonin in seasonal adjustments of glucose homeostasis and allocation to and from the hibernation energy store in bats.

Hibernating bats provide unique potential to test hypotheses about the role of melatonin and photoperiod on hibernation physiology because of natural variation in the photoperiod signals they experience both within and among hibernacula. In contrast to the underground burrows of most rodent hibernacula, sites used for hibernation by bats can vary dramatically in the strength of photoperiod cues. Even within a given species, some bats will roost nearer the entrances of hibernacula than others and may experience a relatively strong light–dark cycle while others will experience weaker cues. Some hibernacula are extremely dark and bats may roost deep underground, thus experiencing little if any photoperiod cue. This natural variation in light–dark stimulation experienced by bats provides an interesting opportunity to examine the role of pineal control of hibernation physiology and behavior. For example, melatonin levels rise with the onset of periodic arousals in hamsters (Darrow et al., 1986) but it is unknown if light levels in the hibernaculum influence the magnitude of these increases.

WNS could lead to a number of changes in melatonin signaling for bats. Bats with WNS roost closer to entrances of caves as the disease progresses (Langwig et al., 2012). If secretion of melatonin depends on dark exposure during arousals, this change in behavior could lead to pronounced changes from normal melatonin levels. Given that melatonin may play a role in glucose and fat homeostasis in bats during their prolonged winter fast (Srivastava and Krishna, 2010), reduced melatonin expression could have direct implications for energy balance. Melatonin could also play a role in arousal timing although its influence for scheduling arousals is not known. Hibernating bats from relatively warm winter climates (e.g., southwest England) synchronize periodic arousals with sunset, warming up at about their normal foraging time throughout hibernation because food may be available on some winter nights, (Hope and Jones, 2012; Park et al., 2000). In cold climates (e.g., central Manitoba, Canada), little brown bats in mid-hibernation do not synchronize arousals with sunset, presumably because foraging opportunities are extremely unlikely (Czenze et al., 2013). Interestingly, though, as spring approaches they reinstate a daily rhythm to arousals and re-warm at their normal foraging time like bats from warmer climates, perhaps because the chance of foraging improves as spring approaches (Czenze, 2013). If these patterns of arousal can be entrained and reinforced by photoperiod, stronger photoperiod cues for WNS-affected bats near hibernaculum entrances might increase the chance of arousing and emerging at night when there is at least some chance to forage. This may be unlikely to help bats survive WNS in northern parts of their ranges with long winters, but could be valuable at relatively southern sites where insects might be available earlier in the year. Given patterns of increased melatonin secretion observed soon after the onset of arousals, greater arousal frequency could further augment melatonin and also influence energy balance by affecting gluconeogenesis, glucose metabolism, lipid metabolism and insulin resistance as suggested by Srivastava and Krishna (2010). On this basis, we predict that bats affected by WNS may have elevated levels of melatonin during the later stages of infection as they reduce clustering and begin to

synchronize arousals with the dark phase. Melatonin secretion appears to be a heritable trait (Hallam et al., 2006; Zarazaga et al., 1998) so, if WNS selects for phenotypic consequences of melatonin expression (e.g., altered glucose homeostasis during fall fattening or late hibernation), we also predict detectable differences in melatonin secretion and sensitivity between pre- and post-WNS bat populations.

Glucocorticoids

The hypothalamic-pituitary-adrenal (HPA) axis regulates the release of glucocorticoids (GCs), the steroid hormones underlying the physiological stress response. The HPA axis triggers secretion of corticotropin-releasing hormone that acts on the anterior pituitary gland, prefrontal cortex and amygdala (McNally and Akil, 2002; O'Connor et al., 2000). This stimulates hydrolysis of pro-opiomelanocortin to β-endorphin and adrenocorticotropic hormone (ACTH), which prompts the release of glucocorticoids from the adrenal glands (O'Connor et al., 2000). In general, the primary consequence of an increase in GCs is catabolic, mobilizing energy reserves, presumably to facilitate a response to a subsequent stressor (Romero, 2002). However, high levels of baseline GCs may also be anabolic, particularly for fat-storing hibernators during the pre-hibernation season (Reeder et al., 2014a) and can affect energy balance via influence on other systems such as inhibition of reproductive behavior (Romero, 2002) and inhibition of immune responses (O'Connor et al., 2000; Reichardt et al., 2001).

Two categories of individual and temporal variation in GC levels are relevant to hibernation behavior and physiology. First, baseline levels of GCs may vary seasonally and among individuals, which could influence the allocation of resources to reproduction versus pre-hibernation fattening (Romero, 2002). Second, individual and seasonal variation in the magnitude of shorter-term (i.e., minutes to hours) fluctuations in GCs could affect seasonal energetics and the energetics of torpor and arousal. Such short-term increases can occur in response to an acute environmental stressor, such as a predation attempt or experimental restraint.

Seasonal variation in baseline GC levels occurs for many species, although patterns of seasonal variation differ among taxa, possibly due to differences in life history (Romero, 2002). Baseline GC levels have been measured in a few bat species (Reeder et al., 2004a, 2004b; Widmaier and Kunz, 1993). In little brown bats, the lowest levels were observed during summer and the highest levels during hibernation and pre-hibernation (Gustafson and Belt, 1981; Reeder et al., 2004a). This could reflect a metabolic role for baseline GCs. In general, GCs increase availability of circulating glucose and triglycerides by stimulating gluconeogenesis from amino acids in the liver, by inhibiting glucose uptake from tissues other than brain and, especially relevant to hibernation, by encouraging lipolysis. These functions could be especially important during pre-hibernation to encourage fattening and, in the midst of hibernation, to preserve glucose in the circulation to fuel metabolism in the brain during torpor and active metabolism (Gustafson and Belt, 1981; Reeder et al., 2004a). Glucocorticoids can also suppress reproductive behavior and function (Rivest and Rivier, 1995; Whirledge and Cidlowski, 2010). Mating occurs during fall and early winter so male bats face a trade-off during pre- and early hibernation between investing in survival (i.e., energy accumulation during pre-hibernation, energy savings via torpor after hibernation onset) or mating behavior. Therefore, variation among males in baseline GCs during the pre-hibernation/early hibernation period could influence allocation of resources to either mating or energy savings and lead to variation in mating behavior.

The acute response of GCs to physiological stress could be especially important in the midst of hibernation. Popova and Koryakina (1981) observed negligible changes in stress reactivity upon arousal from torpor in hibernating red-cheeked ground squirrels (*Spermophilus erythrogenys*). However, arousals may represent a significant physiological stress for bats and glucocorticoids may influence metabolic fuel allocation during arousals (Kwiecinski and Damassa, 2000). Fat is the primary source of energy during arousals, but in big brown bats (*Eptesicus fuscus*) evidence of gluconeogenesis from tissue protein during periodic arousals suggests an effect of glucocorticoids on muscle catabolism. This source of energy is likely to become especially important as fat reserves decline toward the end of hibernation (Kwiecinski and Damassa, 2000; Yacoe, 1983). Arousals themselves may cause physiological stress for bats and disturbance (e.g., by potential predators or human visitors to bat hibernacula) can represent an acute environmental stressor. Differences between natural- and disturbance-induced arousals have recently been observed in golden-mantled ground squirrels (*Callospermophilus lateralis*) with sudden disturbance-induced arousals characterized by faster maximum rewarming rates (Utz and van Breukelen, 2013) possibly as an adaptation to predation risk during hibernation. Hibernating ground squirrels in enclosed burrows may seem unlikely to encounter predators but badgers (*Taxidea taxus*), for example, can represent a significant predation pressure during hibernation (e.g., Michener, 2000). Many bat hibernacula are highly accessible by a range of potential predators from mustelids to rodents. Maintaining an active stress response throughout hibernation and remaining prepared for sudden disturbance could be especially important to help bats respond to potential predation risk within hibernacula.

Glucocorticoid levels could provide insight into the pathophysiology of WNS in bats because of their direct link to stress responses. As for leptin and melatonin systems, the HPA axis also has potential to undergo selection in response to WNS. The increased frequency of arousals and depletion of fat stores associated with WNS may be both a cause and a consequence of increased HPA activity. Disease is an acute environmental stressor (Romero, 2002) and it is possible that WNS causes increased arousal in a similar way that disturbance causes arousal (Utz and van Breukelen, 2013; Wilcox et al., 2014). Czenze et al. (2013) also proposed the hypothesis that some of the increase in arousal frequency observed for bats in WNS-positive hibernacula could reflect disturbance by other individuals. In this scenario, the disease leads to an initial increase in arousal frequency for severely infected bats which, in turn, increases disturbance of roost-mates. In effect, this could generate a positive feedback cycle of disturbance and increasing arousal frequency. Presumably, if disturbance from normothermic conspecifics in the hibernaculum represents an environmental stressor, GC levels will be affected. This hypothesis predicts that bats with WNS should exhibit increased GC levels beyond those normally seen in healthy, undisturbed hibernating bats and perhaps similar to bats frequently disturbed by predators (or researchers mimicking predators). This also predicts that at least part of the explanation for increased arousal frequency in bats with WNS reflects a heightened physiological stress response.

Glucocorticoids might also influence the healing and recovery process for the small proportion of bats that survive WNS, particularly if the disease represents a chronic stressor. Chronic elevation of GCs reduces immune function and inflammatory response (O'Connor et al., 2000; Reichardt et al., 2001). NF-κB is a protein that controls DNA transcription and is key in regulating immune system responses (Li and Verma, 2002; O'Connor et al., 2000). High glucocorticoid concentrations can disrupt normal functioning by binding to activated NF-κB and up-regulating inhibitory proteins, which affects local inflammatory reactions (O'Connor et al., 2000). Immune responses are significantly down-regulated during hibernation in healthy individuals of the bat species most adversely affected by WNS (Moore et al., 2013). Sadly, however, infected individuals that make it through the tight winter energetic bottleneck imposed by WNS, exhibit a rapid re-establishment of immune function in the spring, manifesting in immune reconstitution inflammatory syndrome (IRIS, Meteyer et al., 2012). As their immune function is restored in spring and begins to combat the fungal infection, the rapid neutrophilic inflammatory response can, paradoxically, cause severe negative pathology and likely mortality for some individuals (Meteyer et al., 2012).

This IRIS response seems to be an especially insidious consequence of the disease, given that such an intense spring immune response is probably overkill. The high T_b of active bats in spring could be sufficient to fight off the disease on its own because *Pd* cannot grow at the warm T_b of normothermic bats (Verant et al., 2012). The effect of IRIS may also extend effects of WNS further into the reproductive season. Bats in WNS-impacted areas that suffer from IRIS after emergence may be forced to delay reproduction due to the energetic costs of recovery. Delayed parturition has been documented in WNS-impacted populations (Francl et al., 2012), and young-of-the-year in these populations may have less time to store fat before they enter hibernation, potentially reducing their probability of surviving their first winter (Frick et al., 2010b). Given that high levels of GCs inhibit immune and inflammatory responses, and may inhibit the occurrence of IRIS, we hypothesize that bats with relatively high GC levels in early spring will have a reduced likelihood of IRIS, a greater likelihood of survival through the spring and summer, and potentially greater reproductive success. If high springtime levels of GCs are heritable, this trait could be subject to selection by WNS.

If relatively high spring levels of GCs are beneficial for combatting IRIS, these levels would need to exhibit an upper limit and decline quickly as healing occurs because chronically high GCs in spring could themselves have negative reproductive consequences. Although pregnancy may be supported by the beneficial effect of baseline glucocorticoids for mass gain (Dallman et al., 2000), chronically elevated GCs will inhibit reproductive function and further delay parturition (Rivest and Rivier, 1995; Whirledge and Cidlowski, 2010). Delayed parturition could also have implications for population sex ratios. Mechanisms influencing sex ratios of bats are not known but glucocorticoids have been found to influence sex ratio in other birds and mammals. For instance, high corticosterone levels in white-crowned sparrows (*Zonotrichia leucophrys*) and Japanese quail (*Coturnix japonica*) were associated with female-biased clutches (Bonier et al., 2007; Pike and Petrie, 2006), and high cortisol levels in Richardson's ground squirrels during gestation led to male-biased litters (Ryan et al., 2012). Barclay (2012) showed that female big brown bats that gave birth later in the spring tended to give birth to males. This is presumably because some females may mate in their first year, while males do not, so having female pups early allows for these pups to be reproductively active by autumn (Barclay, 2012). Therefore, if WNS causes chronic, prolonged elevation of GC levels for surviving mother bats during spring, we predict a delay in parturition dates, leading to reduced juvenile survival and a male-biased juvenile sex ratio.

Conclusions

The physiological adjustments influencing energy balance and thermoregulation that must occur before, during and after hibernation result from precise regulation of neuroendocrine activity. Although relatively few studies have focused on bats as model taxa for neuroendocrinological research, they represent an exciting avenue to investigate the influence of hormone systems on hibernation and seasonal energy balance. Studying hormone expression in small hibernators like bats is not without challenges. For instance, hibernators must be followed throughout hibernation to quantify temporal variation because baseline levels of many hormones change seasonally (Romero, 2002; Wang, 1982). Sampling efforts must also be designed to minimize disturbance during hibernation that could affect hormone levels, for instance sampling via catheter (Tøien et al., 2001; Zanella and Mendl, 1992). Torpor expression and neuroendocrine function may vary between the lab and field which must be considered when drawing inferences from laboratory studies. Improvements in techniques for tracking bats in the wild and housing bats naturally in captivity will help to address these issues.

We have focused on three hormone systems – leptin, melatonin and glucocorticoids – in part because their effects on mammals are linked to both hibernation and reproductive phenology and because there are some data available for bats. These three systems may also affect survival from WNS by influencing pre-hibernation fattening, arousal timing and metabolism during hibernation, and immune function and healing responses during and after spring emergence. However, by no means are we suggesting this represents an exhaustive list of systems that might affect hibernation in mammals or differential survival of bats in the face of WNS. Other potentially important examples include (but are not limited to) oxytocin, androgens, vasopressin and thyroid hormones. Wilcox et al. (2014) recently reported that bats with WNS reduce clustering behavior during hibernation, which could reflect effects of oxytocin on social behavior (e.g., Lim and Young, 2006). Androgens may influence mating behavior of bats during hibernation based on the observation that orchidectomized males did not attempt to mate (Mendonça et al., 1996). Frequency of copulation attempts by males has obvious implications for hibernation energy balance in WNS-positive hibernacula where males must choose whether to allocate limited fat reserves to mating attempts or survival. Androgens block daily torpor in Siberian hamsters and could influence pre-hibernation energetics and fat accumulation, as well as mating behavior and energy balance during hibernation. Fluid loss and dehydration have been implicated in the pathophysiology of WNS (e.g., Cryan et al., 2013; Warnecke et al., 2013) and vasopressin could provide clues about disease processes and variation in survival, given its role in regulating osmoconcentration and water balance (McCormick and Bradshaw, 2006). Thyroid hormones influence metabolic rate and T_b regulation in many animals, block torpor expression in at least some winter-acclimatized mammals, and may also impact reproductive function (Anselmo et al., 2004; Kim, 2008; Murphy et al., 2012; Warner et al., 2013; Welcker et al., 2013). In little brown bats, thyroid hormones decline to their lowest levels in winter and increase at arousal (Kwiecinski and Damassa, 2000; Magnus and Henderson, 1988), suggesting a possible effect on spring reproduction (although see Burns et al., 1972). In short, there are many hormone systems worth investigating in the context of hibernation in mammals and WNS in bats.

In our view, hibernating bat species have potential as model systems for endocrinological studies investigating mechanisms underlying hibernation physiology in mammals. With hibernating bat populations in eastern North America experiencing such devastating declines from WNS, such endocrinological studies could also fill an essential parallel function from a conservation physiology perspective, to improve understanding of mechanisms underlying mortality and test the potential of bat populations to evolve resistance or tolerance in response to WNS.

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