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Seasonal programming, not competition or testosterone, drives stress-axis changes in a partially-semelparous mammal

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ABSTRACT

Animals must make tradeoffs between reproduction and longevity. This is particularly pronounced in male arctic ground squirrels (*Urocitellus parryii*), that compete aggressively for territories and mates during a three-week breeding season. Breeding males have high rates of severe wounding, high mortality rates, and high free cortisol levels, along with downstream consequences of chronic stress (weight loss, reduced immune function) that appear to contribute to their early death. The elevated cortisol levels are thought to be a result of the intense intrasexual competition. An alternative hypothesis, however, is that the hormonal change is a seasonal adaptation facilitating the tradeoff of immediate competitive advantage at the expense of long-term survival. We tested a two-part hypothesis: first, that elevated free cortisol during the breeding period is a seasonal change that will still occur in the absence of actual competition, and second, that testosterone maintains this increase. We measured plasma cortisol, corticosteroid-binding globulin, and fecal glucocorticoid metabolites in three groups: wild male ground squirrels, captive males prevented from fighting, and captive castrated males. There were no differences amongst these three groups in free and total plasma cortisol, fecal glucocorticoids, or downstream measures of chronic stress. This suggests that high free cortisol and its effects on breeding males are not a consequence of contest competition during the breeding season, but rather a generalized seasonal change. We found no evidence that testosterone plays a role in maintaining elevated free cortisol in arctic ground squirrel males.

1. Introduction

Male arctic ground squirrels have a competitive, high-stakes breeding season. They hibernate for an average of eight months a year, followed by an active season which lasts only four months (Buck and Barnes, 1999). Within this narrow timeframe, females produce one litter. Thus, a male's entire yearly (and often lifetime) reproductive success is determined by his breeding effort during a three-week window at the onset of the active season. Males acquire their mates by defending a territory and monopolizing females in that area. If they can successfully do this, they have a high likelihood of fathering the offspring of those females: 71% of females reproduce with the male whose territory they resided in (Lacey and Wieczorek, 2001). However, they must exclude competition by fighting off other males, resulting in high rates of wounding (severe wounding in 69%–100% of males; Gillis, 2003; Delehanty and Boonstra, 2011) and high rates of death (28–48% of males die after breeding; Gillis, 2003; Boonstra et al., 2001b). The males who do survive the breeding season and hibernate again in the fall also have lower overwinter survival than females, perhaps due to the tolls of the breeding season, with 63% (Gillis, 2003) to a maximum of 83% (Hubbs and Boonstra, 1997) of those males dying before reaching the next active season. Hence, the majority of the male breeding population dies annually.

During this period of intense competition, breeding males have higher free cortisol levels than non-breeding males, females, and juveniles (Boonstra et al., 2001a, 2001b). Cortisol, the primary glucocorticoid (GC) in arctic ground squirrels (Boonstra et al., 2001a), is involved in the stress response and energetic tradeoffs. GCs increase during stressful events, allowing animals to deal with the stressor by mobilizing energy reserves (lipolysis, raising blood glucose levels, and inhibiting storage of energy), enhancing cardiovascular response, preventing excessive vasoconstriction, and down-regulating immune function to prevent immune and inflammatory responses from overshooting (Sapolsky et al., 2000). When this is an acute stress response, it occurs on a time scale of minutes to hours. When GCs are elevated for a prolonged period of time (days or longer), along with the continuation of their downstream consequences, it is referred to as “chronic stress.” It is often explained that while the acute stress response is adaptive, chronic stress is an emergent accident of prolonging the acute stress response, and so the effects are pathological and maladaptive (e.g. Sapolsky, 1987; Wingfield et al., 1998; McEwen, 2004; Chrousos, 2009). Male arctic ground squirrels do display these pathologies of chronic stress, with breeding males having significantly more mass loss (Gillis, 2003), lower white blood cell counts, and poorer ability to produce antibodies than non-breeding males (Boonstra et al., 2001b).

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The effects become progressively more severe as the breeding season continues (Delehanty and Boonstra, 2011).

On the other hand, the chronic stress and subsequent physiological changes in breeding males may not be a pathological consequence of their aggressive mating strategy, but rather an adaptive seasonal change. The view of chronic stress as a pathology has been challenged with the assertion that animals do experience chronic stress in nature under many circumstances, and so their physiological responses to it have undergone selection and are adaptive (Boonstra, 2013). Many species have elevated GC levels during breeding periods, which may have adaptive value in inducing energy mobilization, altering reactive behavior, or priming systems for future stressors (Romero, 2002; Moore and Jessop, 2003). In other species, elevated GCs suppress reproduction, and this difference is dependent on the ecological context in which breeding occurs (Wingfield and Sapolsky, 2003). For male arctic ground squirrels, the former strategy is adaptive: they have a very short reproductive window and a high reward for successful competitors. Hence, it is advantageous for them to put every bodily resource they have towards breeding and fighting other males, ultimately trading off other process involved in long-term survival, such as maintaining body mass or immune function. In this way, chronic elevation of GCs during breeding could be an “adaptive stress response” (Boonstra and Boag, 1992; Boonstra et al., 2001b) facilitating this distribution of bodily resources through glucocorticoids' permissive effects on fat catabolism and altering reactive behavior.

Similar tradeoffs during breeding involving the stress axis are seen in semelparous animals, those that breed only once before deterioration of the body and programmed death. Well-studied examples of this are Pacific Sockeye salmon (Donaldson and Fagerlund, 1968, 1970) and several species in the dasyurid family of Australian marsupials (Bradley et al., 1980; McDonald et al., 1981) which have elevated GCs during breeding. The increase in GCs in these species have been shown to be related to the increase in sex hormones and reproductive maturation, not only the challenges they experience during breeding. For example, landlocked Sockeye salmon (*Oncorhynchus nerka*) that only migrate short distances still display increases in GCs after sexual maturation, though they were not exposed to the environmental stressors of their migrating conspecifics (Carruth et al., 2000). In a population with both semelparous and iteroparous chinook salmon (*Oncorhynchus tshawytscha*), semelparous individuals were found to have significantly higher free cortisol than iteroparous ones (Barry et al., 2001). However, gonadectomy reduces GC levels in Pacific Sockeye salmon (Donaldson and Fagerlund, 1970), prevents interrenal (adrenocortical) hyperplasia, and can increase lifespan by years (Robertson, 1961). Both sex hormones and male conflicts affect GC levels in dasyruids. Isolation of *Antechinus stuartii* from other males reduces GC levels (Scott, 1987) and captivity of the brush-tailed phascogale (*Phascogale tapoatafa*) prevents significant increase in GCs during the breeding season as well as preventing death, which normal follows immediately after (Schmidt et al., 2006). Yet, sex hormones also have a direct relationship to elevated GCs: castration of *A. stuartii* reduces free GCs by allowing an increase in corticosteroid-binding globulin (CBG) levels, whereas testosterone injections decrease CBG levels (Bradley et al., 1980). Though testosterone and CBG levels are not correlated across individual arctic ground squirrels (Boonstra et al., 2001a; Delehanty and Boonstra, 2011), breeding males have lower CBG levels than those of non-breeding males, and the latter also have lower testosterone levels (Boonstra et al., 2001b; Buck and Barnes, 2003). Thus, in breeding male arctic ground squirrels, high testosterone may cause elevated free cortisol levels by driving down CBG production.

If elevated cortisol during the breeding season is adaptive, rather than pathological, in male arctic ground squirrels, the increase in cortisol should be present as a seasonal change in reproductive males even when they are removed from the actual stress of male-male aggression. To test this hypothesis, we captured and separated newly emerged breeding males prior to fighting. If their cortisol levels do not differ from wild males who are exposed to the stress of the breeding season, this would provide evidence that the stress response during the breeding season is anticipatory, rather than a consequence of competition. Furthermore, if environmental stressors do not trigger this chronic stress response, a potential candidate for regulating this change is the sex hormones, which are in higher concentrations in breeding animals and appear to permit the increase in GCs during breeding in Sockeye salmon and *Antechinus*. Male arctic ground squirrels are known to have increased androgen levels during the peak of the breeding season and also following encounters with other males during spring breeding (Buck and Barnes, 2003). To examine this relationship, an additional group of captive males was castrated, and plasma cortisol and fecal cortisol metabolite (FCM) levels were compared with those of wild males and captive-intact males.

2. Methods

2.1. Trapping and handling

Male arctic ground squirrels (n = 20) were captured at emergence at the Yukon Wildlife Preserve, located approximately 30 km west of Whitehorse, Yukon Territory. They were trapped with Tomahawk Live Traps (Tomahawk, WI, USA) baited with peanut butter. Trapping occurred on April 7th and 8th, 2015, at the onset of the breeding season. Males emerge from hibernation before females (McLean and Towns, 1981), so we were confident that males had just recently emerged, as only 8 of 28 of trapped animals were female. In contrast, the sex ratio once females have emerged is 1:1 (Boonstra et al., 2001b). At capture, squirrels were tagged with monel ear tags (National Band and Tag, Newport, KY, USA) and weighed. Squirrels were briefly anesthetized using a nose cone with cotton soaked in isoflurane (Benson Medical Industries Inc., Markham, ON, Canada) and a 0.3 mL blood sample was taken from the suborbital sinus. Fecal samples were collected from traps. These represent baseline GC levels of individuals in their wild state, as feces reflects plasma cortisol levels 7 h prior to the stress of capture (Bosson et al., 2009). Within 48 h of trapping, animals were transported by vehicle to Kluane Lake Research Station, Yukon, where they were kept in captivity for four weeks. Squirrels were kept indoors in polypropylene cages (47 cm × 26 cm × 20 cm) with mesh lids, in natural light provided by a bank of windows and under ambient temperature. Cages were spaced about a foot apart and were opaque, so squirrels had no visual contact with one another. A wooden board was placed over half of the cage to provide a fully covered area, and cedar bedding and cotton nesting material was provided. Water and food was available ad libitum. Squirrels were fed apple slices, lettuce, and a mixture of Step Right Premium “Classic Sweet” feed and “Complete Pellet” feed (Hi-Pro Feeds, Okotoks, AB, Canada). This feed was composed of grains and molasses with a minimum of 12% crude protein, 2.5% crude fat, and a maximum of 9% crude fiber for the former feed and 14% crude fiber for the latter. All animal care procedures were approved by the University of Toronto Animal Use Committee (protocol 20010861).

2.2. Surgery and sample collection

On April 10th, animals were randomly assigned to the two captive treatments. Half were castrated (n = 10) and a 0.3 mL blood sample was collected from the suborbital sinus. They were anesthetized using 3.5% isoflurane delivered from a portable anesthetic delivery unit and injected with a ketoprofen analgesic (Anafen, Merial, Baie-d'Urfé, QC, Canada). Testes were removed and vas deferens and scrotum were sutured shut. Captive animals to be left intact (n = 10) were also anesthetized in the same manner and a blood sample was taken. One animal from the intact group died under anesthesia. All other animals recovered rapidly from surgery without further complications. Following surgery, blood and fecal samples were collected on a weekly basis (April 17th, April 25th, May 1st, May 8th). On the days of sampling, starting