Hormones and Behavior 56 (2009) 169–176

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Testosterone treatment diminishes sickness behavior in male songbirds

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ARTICLE INFO

Article history:
Received 10 February 2009
Revised 2 April 2009
Accepted 3 April 2009
Available online 15 April 2009

Keywords:
Challenge Hypothesis
Corticosterone
Immune-endocrine interactions
Immunocompetence Handicap Hypothesis
Lipopolysaccharide
Territorial aggression
Testosterone
White-crowned sparrow
Zonotrichia leucophrys gambelii

ABSTRACT

Males of many vertebrate species are typically more prone to disease and infection than female conspecifics, and this sexual difference is partially influenced by the immunosuppressive properties of testosterone (T) in males. T-induced immunosuppression has traditionally been viewed as a pleiotropic handicap, rather than an adaptation. Recently, it has been hypothesized that suppression of sickness behavior, or the symptoms of infection, may have adaptive value if sickness interferes with the expression of T-mediated behaviors important for male reproductive success. We conduct a classic hormone replacement experiment to examine if T suppresses sickness behavior in a seasonally-breeding songbird, Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Triggered experimentally by bacterial lipopolysaccharide (LPS), sickness behavior includes decreased activity, anorexia, and weight loss. Gonadectomized (GDX) males that were treated with silastic implants filled with T exhibited suppression of behavioral and physiological responses to LPS compared to GDX and sham-GDX controls given empty implants. Sickness responses of control groups were statistically indistinguishable. T-implanted birds had significantly higher plasma T than control groups and levels were within the range associated with aggressive interactions during male-to-male contests. These findings imply that suppression of sickness behavior could occur when T is elevated to socially-modulated levels. Alternatively, it is possible that this suppressive effect is mediated through a stress-induced mechanism, as corticosterone levels were elevated in T-implanted subjects compared to controls. We propose that males wounded and infected during contests may gain a brief selective advantage by suppressing sickness responses that would otherwise impair competitive performance. The cost of immunosuppression would be manifested in males through an increased susceptibility to disease, which is presumably offset by capitalizing upon limited reproductive opportunities.

Introduction

Sex differences in disease resistance and immune function are commonplace in vertebrates (Poulin, 1996; Klein, 2000; Nunn et al., 2009) as well as some invertebrates (Kurtz et al., 2000; Nunn et al., 2009). In general, males are more susceptible to disease and have weaker immune responses than female conspecifics (Poulin, 1996; Zuk and McKean, 1996; Klein, 2000; Nunn et al. 2009). On an evolutionary level, these differences are often linked to divergent reproductive strategies between the sexes. Males classically invest heavily in mating effort at the expense of self-maintenance while females place the majority of their reproductive effort into gamete investment and parental care (Andersson, 1994). Furthermore, the fitness benefits of winning contests in males are typically high, predisposing participants to fight, which leads to injury and potential infection (Zuk, 1990).

In vertebrates, sexual differences in disease susceptibility on a proximate level have been partially attributed to the suppressive effects of the steroid hormone testosterone (T) upon the immune system in males (Alexander and Stimson, 1988; Zuk, 1990; Hillgarth and Wingfield, 1997; although see Roberts et al. 2004 for a meta-analysis that finds equivocal support for T suppressing immune function). T-induced immunosuppression has traditionally been viewed as an evolutionary constraint, or handicap, rather than an adaptation. This viewpoint was initially presented as the Immunocompetence Handicap Hypothesis (ICHH), which attempted to explain how androgen-dependent ornaments important for female choice are honestly enforced to signal male quality (Folstad and Karter, 1992). The hypothesis predicted that obligate suppression of the immune system by T (the handicap) would prevent low-quality males from expressing a high-quality trait.

The notion that T suppresses immune function in an obligatory fashion has been challenged by both theoretical and empirical studies. On a theoretical level, obligate immunosuppression is not evolutionarily stable because costs of immunosuppression could be ostensibly circumvented by a mutation that eliminates androgen receptors on

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0018-506X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2009.04.003

immune cells or by conversion of androgens to other metabolites that are not immunosuppressive (Wedekind and Folstad, 1994; Penn and Potts, 1998). Empirically, studies in mammals have generally documented a suppressive effect of T on immune function (Grossman, 1985; Alexander and Stimson, 1988; Schuurs and Verheul, 1990; Nelson and Demas, 1996), although several studies have reported no effects or immunoenhancement (Ansar Ahmed et al., 1985; Olsen and Kovacs, 1996; Bilbo and Nelson, 2001). In birds, T implants suppressed cellular (T-cell) and/or humoral (B-cell) immune responses in some species (Duffy et al., 2000; Evans et al., 2000; Peters, 2000; Casto et al., 2001; Owen-Ashley et al., 2004; Deviche and Cortez, 2005), while no effect was found in other species (Ros et al., 1997; Hasselquist et al., 1999; Roberts et al., 2007). The studies that documented T-induced immunosuppression and measured circulating corticosterone titers found chronically elevated levels (Duffy et al., 2000; Evans et al., 2000; Casto et al., 2001; Owen-Ashley et al., 2004), which also suppress immune function (McEwen et al., 1997; Apanius, 1998). In other species, immunosuppression of cellular immunity is a function of reproductive state and occurs independent of T during the breeding season (Bentley et al., 1998; Greenman et al. 2005). Therefore it remains unresolved whether immunosuppression of cellular and humoral immune responses in male birds is directly regulated by T (Owen-Ashley et al., 2004).

An understudied component of immune function that could provide further insight into an adaptive explanation underlying T-induced immunosuppression is the behavioral response to infection. Most vertebrates initially react to an infectious challenge by exhibiting an array of stereotypical behaviors collectively known as *sickness behaviors*. Triggered by proinflammatory cytokines (e.g., interleukin-1), these behaviors include decreases in food and water intake (anorexia and adipsia, respectively), reduced activity, increased slow-wave sleep, and rapid disengagement from social activities (Hart, 1988; Kent et al., 1992). It has been proposed that the behavioral symptoms of infection are not a deleterious by-product of infection, but rather an adaptive strategy to conserve energy for immune activation, while reducing nutrients in the bloodstream (e.g., iron, zinc) that pathogens require for growth and replication (Hart, 1988). Nonetheless, it should be emphasized that activation and maintenance of sickness behavior (and accompanying acute phase responses) exact considerable energetic and life-history costs (Klasing, 2004; Owen-Ashley and Wingfield, 2007). Because food intake is drastically reduced during sickness behavior, substantial energy reserves are depleted to maintain these responses, as well as the thermoregulatory demands of fever (for those species that exhibit hyperthermia as opposed to hypothermia; Lochmiller and Deerenberg, 2000; Klasing, 2004; Martin et al. 2008). This energetic bottleneck is partially offset by a reduction in activity, which saves energy. From a life-history standpoint, expression of sickness behavior creates an "opportunity cost" by suspending important life-history stages, such as reproduction and growth (Klasing et al., 1987; Owen-Ashley et al., 2006; Owen-Ashley and Wingfield, 2007). Territorial aggression (Cirulli et al., 1998; Owen-Ashley et al., 2006; Owen-Ashley and Wingfield, 2006), parental care (Bonneaud et al., 2003), and sexual behavior (Avistur and Yirmiya, 1999) are curtailed to conserve energy for immune activation. The temporary suspension of these activities is likely mediated by proinflammatory cytokines, which activate sickness behavior and the hypothalamo–pituitary–adrenal (HPA) axis (Bateman et al., 1989), while concomitantly inhibiting the hypothalamo–pituitary–gonadal (HPG) axis (Besedovsky and del Ray, 1996; Avistur and Yirmiya, 1999).

Evidence for T regulating sickness behavior comes from research examining lipopolysaccharide (LPS)-induced sickness responses in captive and wild vertebrates. LPS is a bacterial endotoxin that mimics an infectious inoculation without exposing subjects to real pathogens (Kent et al., 1992). In laboratory mice, castration enhances adrenal and immune responses to LPS stimulation compared to shams and testosterone replacement reverses this effect (Spinedi et al., 1992; Seale et al., 2004). In contrast, castrated Siberian hamsters (*Phodopus sungorus*) suppress sickness responses after LPS treatment compared to gonad-intact males (Prendergast et al., 2008), suggesting that T may have an enhancing effect in this species. In free-living song sparrows (*Melospiza melodia morphna*), males experimentally challenged with LPS reduce territorial aggression and lose body mass during the winter (non-breeding conditions), while breeding males in the spring seemingly fail to exhibit any sign of illness (Owen-Ashley and Wingfield, 2006). This seasonal difference is partially attributed to energy limitation, as non-breeding males are heavier and have more fat stores than breeding birds that they could afford to lose after becoming anorexic (Owen-Ashley and Wingfield, 2006). However, these results also imply that T could contribute to the suppression of sickness behavior in the spring when levels are elevated.

According to the Challenge Hypothesis, T levels in males of most monogamous songbirds are basal during the winter (Level A), and rise in the spring to a breeding baseline (Level B) sufficient to support spermatogenesis, development of some secondary sexual characteristics and accessory organs, as well as reproductive behavior (Wingfield et al., 1987; Wingfield et al., 1990). Peak levels (Level C) are achieved through social stimulation from male–male territorial contests and/or mate-guarding when reproductive potential typically is the highest (Wingfield et al., 1987; Wingfield et al., 1990). Competing over access to mates involves risks, and fighting can lead to injury and subsequent infection (Zuk, 1990; Andersson, 1994). A strong sickness response to an infected wound during these contests would likely lead to reduced aggressive behavior and further inhibition of the HPG axis. It is therefore plausible that the temporary suppression of sickness behavior by T could occur if the fitness costs of losing a territory and/or being cuckolded by another male outweighed the cost of delaying a behavioral response to infection.

In this study, we investigated the effect of T on sickness behavior in male Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*), a songbird that breeds at high latitudes (47–68°N) in western North America. Males were castrated to remove gonadal sources of T, photostimulated on long days, and then surgically implanted with a small silastic capsule that was either empty or filled with T. A third group was sham-castrated and given an empty implant to serve as an intact control. Birds were then injected with LPS or saline vehicle, and changes in food intake, body mass, and activity were measured. We predicted that castrated males receiving T implants and intact birds would have higher T levels and therefore exhibit reduced sickness responses compared to castrated controls.

Methods

Gambel's white-crowned sparrows were captured in central Washington at a stopover site (46° 8′ N, 119° 50′W) during their fall migration and housed in outdoor roof aviaries at the University of Washington, Seattle. Food (bird seed and Mazuri bird chow) and water were available *ad libitum* throughout this study. During the fall, birds were sexed by unilateral laparotomy and returned to aviaries. In early February, first-year male white-crowned sparrows were placed in two environmental chambers (approx. $6' \times 10' \times 8'$) set on a short-day photoperiod (8L:16D) at 20 ± 1 °C. A subset of birds ($N = 18$) was bilaterally gonadectomized (GDX). The remaining birds ($N = 7$) were sham-GDX to serve as intact controls. During these procedures, subjects were deeply anesthetized using an isoflurane vaporizer (Summit Medical Equipment Co., Bend, Oregon). All gonadectomies were performed on birds that had regressed gonads (non-breeding condition). The GDX subjects were later used and sacrificed in an unrelated study, and their autopsies confirmed that gonadectomy was complete and successful.

In early March, subjects were moved into individual cages and photostimulated on long day lengths (LD = 20 h light) to mimic the photoperiod experienced on Alaskan breeding grounds. Twenty-five