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Testosterone production and social environment vary with breeding stage in a competitive female songbird

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ABSTRACT

In many vertebrates, males increase circulating testosterone (T) levels in response to seasonal and social changes in competition. Females are also capable of producing and responding to T, but the full extent to which they can elevate T across life history stages remains unclear. Here we investigated T production during various breeding stages in female tree swallows (*Tachycineta bicolor*), which face intense competition for nesting sites. We performed GnRH and saline injections and compared changes in T levels 30 min before and after injection. We found that GnRH-injected females showed the greatest increases in T during territory establishment and prelaying stages, whereas saline controls dramatically decreased T production during this time. We also observed elevated rates of conspecific aggression during these early stages of breeding. During incubation and provisioning, however, T levels and T production capabilities declined. Given that high T can disrupt maternal care, an inability to elevate T levels in later breeding stages may be adaptive. Our results highlight the importance of saline controls for contextualizing T production capabilities, and they also suggest that social modulation of T is a potential mechanism by which females may respond to competition, but only during the period of time when competition is most intense. These findings have broad implications for understanding how females can respond to their social environment and how selection may have shaped these hormone-behavior interactions.

1. Introduction

Hormones modify suites of traits in response to changing environmental conditions, and in doing so, they allow animals to respond flexibly to their environment (Adkins-Regan, 2005; Wingfield, 2015; Zera et al., 2007). Social environments can be especially dynamic and thus necessitate hormonal mechanisms to appropriately modulate behavior. In male vertebrates, several lines of evidence suggest that the steroid hormone testosterone (T) may coordinate these behavioral responses to dynamic social environments. For example, males typically produce more T during early breeding stages that involve more competition and mating effort, but less T when social competition is less pronounced, e.g. during periods of parental care (Goymann, 2009; Klukowski and Nelson, 1998; Muller and Wrangham, 2004; Wingfield et al., 1990). In addition, in some species, males temporarily elevate T levels in response to aggressive challenges from another male (Archer, 2006; Oliveira, 2009; Teles and Oliveira, 2016; Wingfield, 1985- but see Goymann, 2009), further suggesting that circulating T levels are coupled with aggression during periods of social competition, at least for males (Wingfield, 2017; Wingfield et al., 1990).

Female competition for resources and mates is likewise widespread, but less is known about the hormonal mechanisms that allow females to respond to social instability (Clutton-Brock, 2009; Petty and Drea, 2015; Rosvall, 2013b; Stockley and Campbell, 2013). One possibility is that, like males, females can match T levels to their social environment, such that female competition and aggression are coordinated by changes in T production. Vertebrate ovaries are capable of producing and secreting T (Geniole et al., 2017; Goymann and Wingfield, 2014; Ketterson et al., 2005; Staub and De Beer, 1997). In turn, T in circulation may influence female behavior via conserved neural circuits (Duque-Wilckens and Trainor, 2017; Staub and De Beer, 1997). However, direct tests of whether females can elevate circulating T in response to social challenges are limited, and results are mixed: In some species, females elevate T levels in circulation in response to fights or in relation to the density of conspecifics (e.g., Bateup et al., 2002; Desjardins et al., 2006; Gill et al., 2007), but in other species, females do not appear to elevate T, and may even decrease T in circulation in response to competition (Duque-Wilckens and Trainor, 2017; Rosvall, 2013b). To interpret these mixed results and begin to understand how and why females respond to social competition in the ways that they do, it becomes critical to understand the full extent of their T production capabilities.

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GnRH challenges – or injections with gonadotropin-releasing hormone (GnRH) – are useful assays for exploring an individual's ability to produce T. GnRH is the peptide hormone naturally produced by the hypothalamus in response to changing environmental conditions, including lengthening days or social stimulation (Adkins-Regan, 2005). This hormone stimulates the hypothalamo-pituitary-gonadal (HPG) axis, which results in gonadal production and secretion of sex steroids, including T. By administering a standardized dose of exogenous GnRH, researchers can essentially flood the HPG axis, allowing for roughly maximum trophic hormone release from the pituitary, and production and secretion of gonadal steroids. Thus, GnRH challenges are thought to provide a measure of the upper limits of an individual's potential hormonal response at a given time (Jawor et al., 2006). Prior research has demonstrated that the degree to which males can increase circulating T in response to GnRH differs among individuals and is consistent within an individual (Jawor et al., 2006; Needham et al., 2016). Thus, GnRH challenges are useful for characterizing individual differences in hormonal phenotypes, particularly in the wild where baseline T levels may fluctuate in relation to variable environmental conditions (Kempenaers et al., 2008). Furthermore, T response to GnRH challenges have been found to correlate with aggression, body size, parental care, breeding success, and social status (Bradley and Stoddart, 1997; Cain and Pryke, 2016; Mcglothlin et al., 2008), providing strong evidence that the hormonal phenotypes revealed by GnRH challenges are relevant to individuals' behavior and reproductive success.

Though fewer GnRH challenges have been conducted on females than males, there is some evidence that females can elevate T following GnRH injections (e.g., Jawor et al., 2007; Peluc et al., 2012). Moreover, this ability to elevate T is not limited to breeding stages involving egg production (Covino et al., 2018; Devries et al., 2011; Rosvall et al., 2013), and elevated T levels correlate with aggression and parental effort (Cain and Ketterson, 2012, 2013), suggesting that T production in females may play a role in behavioral regulation. However, some studies report that females do not appear capable of elevating T levels in response to exogenous GnRH (Ambardar and Grindstaff, 2017; Goymann and Wingfield, 2004; Spinney et al., 2006). There are several possible biological explanations as to why females may be capable of modulating T levels in some circumstances but not in others. For example in males, trade-offs between mating effort and parental effort may favor diminished T signaling, especially when elevated T negatively affects parental care and offspring success (Lynn, 2008). In principle, these constraints also should shape hormonal mechanisms in females, such that females would face steep costs if they were to couple T and behavior during parental phases of reproduction (Ketterson et al., 2005; Okabe et al., 2013; Rosvall, 2013a). While only a few studies have performed GnRH challenges in females during multiple breeding stages (e.g., Jawor et al., 2007) and others have looked for relationships between T and aggression within particular breeding stages (e.g., Cain and Ketterson, 2013; Devries et al., 2015), there is still a need for studies that examine seasonal changes in females' ability to produce and secrete T in the context of the changing demands of female competition and parental care.

In some cases, methodological differences may explain the varying results of GnRH challenges in females, such that females *appear* incapable of elevating T in response to GnRH when they are in fact capable. GnRH challenges unavoidably involve significant handling and restraint stress, which, at least in males, is known to substantively decrease circulating T in the absence of additional GnRH stimulation (Deviche et al., 2010). Thus, it is possible that some degree of GnRH-induced T production can be masked by stress-induced T depletion or suppression. This may be especially relevant when T levels are constitutively low, as is often the case for females. The best way to account for any stress-induced decreases in T during GnRH challenges is to compare GnRH-induced changes in T (ΔT) to saline-induced ΔT, which may well be negative. This practice is not common in studies using GnRH challenges (e.g., Ambardar and Grindstaff, 2017; Jawor et al., 2007; Rosvall et al., 2013-but see Covino et al., 2017; Covino et al., 2018; Devries et al., 2011), perhaps because GnRH challenges are often used as an bioassay of an individual's endocrine phenotype, and comparisons among individuals do not necessarily warrant the same control comparisons. These controls become critical, however, for interpreting the magnitude of GnRH-induced T elevations at a particular stage or time. Without proper controls across different breeding stages, we are left without a clear understanding of what the null expectations for responses to GnRH injections should be at that time (i.e., $\Delta T = 0$ vs. $\Delta T < 0$), and therefore cannot properly measure seasonal variation in the magnitude of female T production capabilities.

Tree swallows (*Tachycineta bicolor*) are an ideal system for examining how a female's ability to elevate T may change across breeding stages with varying levels of social instability and parental demands. They are single-brooded, secondary cavity-nesting songbirds, which mean that their reproductive output is dependent upon obtaining a suitable nesting site that they cannot excavate for themselves. Females therefore compete intensely for access to cavities, which are oftentimes artificial cavities or nest boxes. When nesting sites are limited, more aggressive females are more successful at obtaining boxes than less aggressive females (Rosvall, 2008). Floater populations, which are predominantly made up of young females that have not yet obtained boxes, frequently investigate occupied nesting sites throughout the breeding season (Stutchbury and Robertson, 1987a), and have been known to evict breeding females from their boxes (Leffelaar and Robertson, 1985). Thus, competition for boxes is most intense in the early stages of territory establishment and nest-building, but the threat of social challenges persists throughout the entire breeding season. Short-term elevations in T are one potential mechanism by which females could respond to these social challenges, supported by the fact that female tree swallows become more aggressive in response to exogenous T (Rosvall, 2013a). However, experimentally elevated T also greatly diminishes incubation behavior in this species (Rosvall, 2013a), suggesting that elevations in T may be especially costly to females during breeding stages involving parental care.

Therefore, based on evidence that selection may favor mechanisms that prevent T from disrupting parental care when it is especially critical for offspring survival (Lynn, 2008), we hypothesized that females would vary seasonally in their ability to produce T alongside seasonal changes in social competition and parental care. To test this hypothesis, we performed GnRH challenges on female tree swallows over consecutive stages of their breeding season: territory establishment/nest-building, pre-laying (yolk formation), egg laying, incubation, and chick provisioning. By comparing changes in circulating T between GnRH-injected females and control (saline-injected) females, we were able to detect any slight elevations that might otherwise be masked by stress-induced reductions in circulating T. To begin to place our T results in the context of female aggressive behavior, we also conducted behavioral observations to quantify the frequency of conspecific fighting throughout the breeding season. We predicted that females would be most able to elevate T early in the breeding season, when competition is most intense, and that females' T-producing abilities would diminish as social competition decrease and the demands of parental care increase.

2. Methods

2.1. Study system and capture techniques

All subjects were female tree swallows captured at artificial nesting cavities (nest boxes) located around Bloomington, IN, USA (39°9 N, 86°31 W) between March 23 and June 18, 2016. We checked nest boxes every few days to monitor nest construction, egg laying, hatching, and fledging. Subjects were captured in their nest boxes between 07:00 and 13:00 EDT, by setting nest box traps (Stutchbury and Robertson, 1986) or by covering box entrances while birds were already inside. We visually monitored boxes to ensure that birds were not trapped for