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Up to the challenge? Hormonal and behavioral responses of free-ranging male Cassin's Sparrows, *Peucaea cassinii*, to conspecific song playback

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ABSTRACT

The Challenge Hypothesis postulates that male vertebrates can respond to social challenges, such as simulated territorial intrusions, by rapidly increasing their concentrations of plasma androgens, such as testosterone (T). This increase may facilitate the expression of aggressive behavior and lead to persistence of this behavior even after withdrawal of the challenge, thus potentially promoting territoriality and the probability of winning future challenges. The scope of the Challenge Hypothesis was tested by exposing free-ranging male Cassin's Sparrows, *Peucaea cassinii*, to conspecific song playback (SPB) at the beginning of the vernal nesting season. Exposure to SPB stimulated aggressive behavior but did not influence plasma T. Furthermore, plasma T did not correlate with the duration of exposure to SPB, and the behavioral response to SPB did not differ in males that were challenged a second time shortly after the first challenge. As birds were investigated at a stage of their reproductive cycle when plasma T is presumably seasonally high due to photostimulation, the lack of hormonal response to SPB may have been due to the hypothalamus–pituitary–gonadal axis secreting hormones at maximum rates. This was not the case, however, because administration of gonadotropin-releasing hormone I rapidly stimulated the secretion of luteinizing hormone (LH) and T, and treatment with ovine LH rapidly stimulated T secretion.

Introduction

In many birds the activity of the hypothalamus–pituitary–gonadal (HPG) axis changes seasonally under the influence of photoperiod (Wingfield et al., 1997) and is facultatively modulated by non-photoperiodic factors that include social interactions (Wingfield et al., 1987, 1990). The influence of social factors on the HPG axis is suggested by correlative studies of territorial male birds indicating that plasma testosterone (T) is often seasonally highest at the beginning of the breeding season, when male–male interactions are frequent and females are sexually receptive (Wingfield et al., 1987), and is confirmed by experimental studies. One commonly used experimental procedure is to expose individuals to simulated territorial intrusions (STI) consisting of pre-recorded conspecific vocalizations combined with a live (Charlier et al., 2009; Hau and Beebe, 2011; Landys et al., 2007; Moore et al., 2004; Wingfield, 1994a) or stuffed (Apfelbeck and Goymann, 2011; Silverin et al., 2004) decoy. Exposure to STI often elicits a strong aggressive response (Gill et al., 2008; Klukowski and Nelson, 1998; Wingfield, 1994b) and, in some situations, a rapid (within minutes) increase in plasma T (Ros et al., 2003; Wingfield, 1984, 1994b). In free-ranging Song Sparrows, *Melospiza melodia*, exposure to conspecific song playback alone or with a live decoy bird has a similar stimulatory effect on aggressive behavior, but plasma T increases significantly only in sparrows that are exposed to both stimuli (Wingfield and Wada, 1989). Whether visual in addition to auditory stimulation is necessary to rapidly elevate plasma T also in other species has not been investigated.

Testosterone can exert rapid (within minutes to hours) physiological (Sachs and Leipheimer, 1988) and behavioral effects (Lord et al., 2009), and in some situations enhances attention to novel conspecifics and other relevant stimuli (rat, *Rattus norvegicus*: Thor et al., 1982; chicken, *Gallus domesticus*: Archer, 1977). According to the Challenge Hypothesis (Wingfield et al., 1987, 1990), elevated plasma T in response to social challenge may normally function not to stimulate aggression per se, but to enhance the intensity of the behavioral response to STI and the persistence of this response even when an individual is no longer challenged (Oyegbile and Marler, 2005; Wingfield, 1994a,b, 2005), which in turn may promote territoriality (Oliveira et al., 2009).

Social challenges can induce rapid endocrine changes in insects (juvenile hormone: Kou et al., 2008; Tibbetts and Huang, 2010), fish

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(Oliveira et al., 2002; Pankhurst and Barnett, 1993; Ros et al., 2003), reptiles (Rubenstein and Wikelski, 2005), birds (Beletsky et al., 1992; Ferree et al., 2004; Ros et al., 2002; Smith et al., 2005; see above), and mammals (Buck and Barnes, 2003; Setchell et al., 2008) including humans (Fry et al., 2011; van der Meij et al., 2010). However, other studies found no influence of single or repeated social interactions on plasma androgens (birds: Addis et al., 2010; Apfelbeck and Goymann, 2011; Apfelbeck et al., 2011; Gill et al., 2008; lizards: Klukowski and Nelson, 1998; Thompson and Moore, 1992) whereas in the Great Tit, *Parus major*, and Blue Tit, *Cyanistes caeruleus*, plasma T decreased in response to STI (Landys et al., 2007; Van Duyse et al., 2004; reviews: Goymann, 2009; Goymann et al., 2007). To reconcile these apparent discrepancies, it has been proposed that the androgen response to a social challenge is contingent upon factors that include the mating system (Wingfield et al., 1990; single- vs. multiple broodedness: Landys et al., 2007), the contribution of males to incubation (Hirschenhauser et al., 2003), and the duration of the breeding season (Goymann, 2009). As proposed by the latter author, the lack of androgen response to STI in some studies may also reflect the fact that observations were made when the activity of the HPG axis, including T secretion, was at its seasonal highest and physiologically incapable of further activation. This could be the case in males of single-brooded species particularly at the beginning of their breeding season.

The functionality of the HPG axis can be evaluated by measuring hypothalamic gonadotropin-releasing hormone (GnRH-I), the plasma luteinizing hormone (LH) response to administration of GnRH-I or the secretagogue N-methyl-D,L-aspartate (NMA), and the plasma T response to LH (Dawson, 2005; Deviche et al., 2010) or GnRH (Jawor et al., 2006; Spinney et al., 2006) administration. For example, in male Cassin's Sparrows, *Peucaea cassinii*, a NMA injection stimulates plasma LH less when birds are photostimulated and have elevated plasma LH than when they are exposed to short days and have low plasma LH (Deviche et al., 2008). In the Green Anole, *Anolis carolinensis*, lightweight males have lower plasma T than heavyweight males, but GnRH administration does not increase plasma T in either form, suggesting that intact lightweight and heavyweight males are secreting this hormone at the maximum rate (Husak et al., 2009). To our knowledge, only one study has investigated the HPG axis functionality in the context of the Challenge Hypothesis (Apfelbeck and Goymann, 2011). In this study on male Black Redstarts, *Phoenicurus ochruros*, plasma T increased in response to GnRH injection but not STI, suggesting that the lack of hormonal response to a social challenge was not due to physiological incapacity to increase T secretion. Additional studies are warranted to test the generality of this conclusion.

We tested the scope of the Challenge Hypothesis in field experiments using a seasonally breeding, socially monogamous male songbird, the Cassin's Sparrow (Dunning et al., 1999), as an experimental model. Avian studies that investigate this hypothesis typically challenge birds using a combination of acoustic (conspecific song playback, SPB) and visual (decoy bird) stimulation (see above). Here we were interested in determining whether exposure to SPB alone produces hormonal and behavioral changes that are consistent with the Challenge Hypothesis. Sparrows were studied at the beginning of their breeding period, when plasma T in other socially monogamous male songbirds generally is at its highest. To evaluate whether the HPG axis activity at the time of the study was at its maximum, we measured the plasma LH response to acute treatment with GnRH-I, and the plasma T response to a GnRH-I or LH injection. Once exposed to STI, some birds in breeding condition remain highly aggressive even after STI withdrawal (Wingfield, 1994b). The physiological basis of the persistence of this behavior is unknown and may involve a short-term sensitization of the pituitary gland to GnRH and/or of the testes to LH. To address this issue, we quantified the influence of SPB exposure on the sensitivity of the pituitary gland to GnRH or of testes to LH by comparing the effects of GnRH-I or LH treatment described above, prior to and following SPB. We also determined whether the behavioral response to SPB differs between birds exposed to this stimulus for the first or second time.

Materials and methods

Animals

We investigated adult male Cassin's Sparrows at the Comanche National Grasslands (Baca Co., Colorado, USA; 37° 06′ N; 102° 34′ W) between the 14th and 19th of May 2010. The species in the study area is considered migratory, but its secretive habits outside the breeding season limit the amount of information that is available on its migratory patterns (Dunning et al., 1999). During the breeding season, males defend territories mostly through song duels that do not involve physical contact (Dunning et al., 1999). Cassin's Sparrows are thought to be socially monogamous (Dunning et al., 1999) and to establish seasonal breeding territories and, at least in part of their breeding range, to normally be double-brooded (Texas: Schnase et al., 1991). Whether the latter applies to birds in the current study area is, however, conjectural. Males at the time of the study had established territories and were skylarking (=territorial flight singing) at a high rate (personal observations), a behavior that in this species is most often given in the presence of females (Dunning et al., 1999). Three females caught during the study had partially developed incubation patches, indicating that the birds were at an early stage (preincubation) of their reproductive cycle (Dunning et al., 1999). All birds were caught and observed between 0600 and 1600. A previous study on free-ranging males of a conspecific species, the Rufous-winged Sparrow, *Peucaea carpalis*, found that plasma T does not change consistently during the course of the day (Deviche et al., 2012).

The study included 98 males that we randomly assigned to one of three independent experiments (Experiments 1, 2, and 3; see below and Fig. 1). Sparrows in Experiment 1 ($n=48$) and Experiment 2 ($n=32$) were caught in Japanese mist nets in response to SPB; birds in Experiment 3 ($n=18$) were used for a behavioral study but were not caught. Sex was determined based on behavioral observations before capture (only male Cassin's Sparrows sing and skylark; Dunning et al., 1999) and, after capture, confirmed by the presence of a developed cloacal protuberance (males only) or incubation patch (females only; Pyle, 1997).

All activities were authorized by the Colorado State Department of Natural Resources and the US Fish and Wildlife Service, and approved by the Arizona State University Institutional Animal Care and Use Committee.

Experimental designs

Experiment 1

The first objective of Experiment 1 was to determine whether exposure to SPB influences *initial* (="baseline") plasma T. Birds were exposed to SPB for a short (=SHORT SPB) or long (=LONG SPB) duration using a stationary portable system (MP3 player) equipped with multidirectional speakers that were usually placed 0.5–1 m above ground. For exposure to SHORT SPB, a mist net was deployed where a bird was found singing, the SPB system was placed near the net and turned on, and the focal bird was then caught as soon as possible and immediately bled. Males in the SHORT SPB group ($n=24$) were exposed to SPB for 4.1 ± 0.5 (S.E.) min before capture. For exposure to LONG SPB, a similar procedure was followed except that the SPB was turned on for 30 min before the mist net was unfurled and the bird then caught as soon as possible. Males in the LONG SPB group ($n=24$) were exposed to SPB for 36.2 ± 1 min before capture. Within 3 min of capture and removal from the net, a blood sample (=*initial* bleed; 100 μl) was collected from the right jugular vein of each sparrow into a heparinized microsyringe. These samples were used to determine *initial* plasma T concentrations.