Ethology

RESEARCH PAPER

Androgens Increase Persistence But Do Not Affect Neophobia in a Problem-Solving Context in a Songbird

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Abstract

Gonadal steroid hormones enhance cognitive performance, particularly spatial and vocal learning, in mammals and birds. However, it is unknown whether problem-solving ability is similarly regulated. We propose that androgens, such as testosterone and 5α-dihydrotestosterone, play a role in mediating problem-solving behavior as well. As a test, male white-crowned sparrows (*Zonotrichia leucophrys gambelii*) were either castrated and administered a blank (Blank-castrate) or testosterone-filled implant (T-castrate) or were sham operated and were exposed to a novel feeder, which they had to open to receive a food reward, in two trials. Testosterone treatment affected neither a neophobic response nor problem-solving performance. However, T-castrates were more persistent in manipulating the feeder than Blank-castrates or Shams. Furthermore, their persistence correlated positively with circulating levels of both testosterone and 5α-dihydrotestosterone. We suggest that a positive correlation between sex steroids and persistence in foraging and problem-solving contexts may lead to an adaptive increase in resource acquisition in the breeding season. Given the overall low success on the problem-solving test, we cannot confidently conclude that androgens do not play a role in mediating problem-solving behavior. However, unlike in mammals, it seems these hormones do not significantly influence neophobia in foraging contexts in birds.

Introduction

Cognition, the process by which individuals use information, is key to adaptive behavior (Dall et al. 2005), thus likely influences how organisms cope with novel environments. Annual variation in environmental conditions creates seasonal demands for enhanced cognitive performance. Indeed, seasonal plasticity of neural structures in the song control system as well as vocal behaviors has been demonstrated in songbirds (Nottebohm 1981; Nottebohm et al. 1986; Tramontin & Brenowitz 2000). Similar neuroplasticity has been found in the hippocampus of food-hoarding (Barnea & Nottebohm 1994; Smulders et al. 1995, 2000; Clayton & Cristol 1996) and brood parasitic birds (Clayton et al. 1997). However, the hormonal regulation of seasonal plasticity in cognitive function is not well understood, especially in birds.

Circulating levels of gonadal androgens (such as testosterone and 5α-dihydrotestosterone) and estrogen show a remarkable fluctuation with season in seasonally breeding vertebrates (Norris & Carr 2013) and have been suggested to facilitate spatial and vocal learning, as well as memory formation, particularly in mammals and birds (Frye et al. 2004; Oberlander et al. 2004; Pyter et al. 2006; Hodgson et al. 2008; Spritzer et al. 2011; Calisi et al. 2013). Androgens act directly on the hippocampus (Frye et al. 2004) or by aromatization to estrogens (Oberlander et al. 2004). The effects of these steroids are likely mediated through the potentiation of cholinergic neurotransmission in the septo-hippocampal pathway and other related brain areas (Leonard & Winsauer 2011).

Recently, an ecologically relevant cognitive skill, problem-solving ability, has been demonstrated to have fitness consequences and suggested to play a significant role in shaping life history evolution (Cole & Quinn 2012; Cole et al. 2012; Cauchard et al. 2013). Given the ecological and evolutionary significance of problem-solving, it is important to understand the roles steroid hormones play in mediating this trait.

Our objective was to investigate whether androgens play a role in facilitating problem-solving behavior. We tested the hypothesis that elevated levels of androgens improve problem-solving performance. Testosterone (T) implants were administered to manipulate levels of androgens in gonadectomized male Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) during the non-breeding season, a time when endogenous levels of androgens are basal. The task required the subject to open a novel feeder baited with a favorite food item. As problem-solving success may depend on how readily and persistently an individual manipulates the novel feeder, we measured parameters of neophobia as well as persistence during the trials. We predicted that T-implanted birds would (1) more rapidly approach and contact the novel feeder, (2) demonstrate more persistence in manipulating the feeder, and as a result (3) obtain the food faster than Blank-implanted or Sham controls.

Methods

Subjects and Housing

The tests performed here were part of a larger study investigating the role of androgens in the development of migratory behavior (Ramenofsky & Németh 2014). We used 24 adult male white-crowned sparrows captured near Davis, CA, USA (38°31′ 53″N, 121°47′ 11″W) in Nov. 2010. Birds were held in individual cages under natural local photoperiod and were fed *ad libitum* with commercial seed-mix (millet, cracked corn, and sunflower seeds) and Mazuri Small Bird Maintenance Diet supplemented with Health Blue Grit (Seed Factory, Ceres, CA, USA). Fresh drinking and bath water were provided daily.

Surgical Procedures and Testosterone Administration

On Dec. 10, 2010, 16 individuals were castrated bilaterally, while eight individuals were sham operated in which the gonads were manipulated but left intact. All surgical procedures were conducted under general anesthesia with vaporized Isoflurane (Attane, Baxter Co). On Feb. 1, 2011, the 16 castrated individuals were implanted subcutaneously with Silastic tubing (Dow Corning) sealed at both ends with Silastic glue (inside diameter = 1.47 mm, outside diameter = 1.95 mm, length = 10 mm). Eight of the castrates received implants packed with 8 mm of crystalline testosterone (Sigma Chemical), while the other eight castrates received empty implants. The eight Sham castrates were manipulated as the implanted birds but did not receive implants. Implants were removed on Feb. 17, 2011. Effectiveness of the surgeries was tested 1 year later when birds regained photosensitivity. At that time, birds were exposed to 18L:6D for 2 weeks and then autopsied for presence of any testicular tissues. The autopsies revealed three blank-castrates with testicular remnants and one sham bird was identified as a female. These subjects were removed from the study, and the sample sizes per group were adjusted as follows: Blank-castrates: 5, T-castrates: 8, Shams: 7. All procedures were approved by the Institutional Animal Use Committee of the University of California Davis.

Experimental Design

All birds were presented with a novel feeder in their home cage. The feeder was a vertical glass test tube (10 × 75 mm) containing two waxworms (*Pyralis farinalis*) and plugged in with a cotton ball in the bottom. The feeder was centrally placed on the door of the cage and above the grit cup. Subjects were required to land on the grit cup to access the feeder and to extract the cotton ball that allowed the worms to fall into the grit cup. This novel feeder task has been used successfully in the same species in the same time of the year, with 5 of 20 unmanipulated individuals opening the feeder within two trials, suggesting that it is a feasible problem to solve for these birds (Z. Németh and M. Ramenofsky, in prep.). Each bird participated in two trials over two consecutive days (on days 14 and 15 of implant; photoperiod 10.7L:13.3D), and each trial lasted for 1 h. Trials were conducted at the same time in the morning on both days. Waxworms were selected as food reward as they represent a preferred food item that is not part of the regular diet. Birds were given two worms a day for 2 d preceding the first trial to assess whether and how quickly the worm was consumed without the presence of a novel feeder. The worms were presented in the grit cup. All birds, except one T-castrate, showed interest and ate the first worm within 10 s of presentation. As this individual neither interacted with nor ate the worm after 3 h of exposure, it was excluded from the experiment reducing the final group size of T-castrates to 7.

Latency to both approach, by landing on the familiar grit cup, and latency to contact the worms in the absence of the novel feeder were used as baseline measures for the calculation of neophobia (Greenberg & Mettke-Hofmann 2001). Because waxworms are a favored food type, we did not deprive the birds of their regular *ad lib* seed-mix diet during or prior to the trials. Experimenter left the room immediately after installing the novel feeders. Trials were video-recorded and scored by a single observer who was blind to treatment groups. Variables measured included: (1) neophobia-related variables: latency to approach (i.e., land on the grit cup), and latency to contact the feeder; (2) persistence-related variables: number of times the bird approached the novel feeder within 10 min of the experimenter leaving the room in the first trial, and the number of pecks delivered at the novel feeder within 10 min of initial contact; and (3) problem-solving performance-related variables: whether or not the individual was successful in solving the task, and if successful, latency to pull the cotton ball, and latency to eat the first worm were measured.

Androgen Radioimmunoassays

Blood samples were collected 1 d after the last trial (day 16 of implant) and plasma was analyzed for testosterone (T) and 5α-dihydrotestosterone (DHT) with a radioimmunoassay following chromatographic separation on diatomaceous earth/glycol microcolumns after the methods of Wingfield & Farner (1975). Samples were randomized and run in three assays. Samples were spiked with 2000 cpm doses of each labeled steroid to calculate recoveries. Final hormone values were adjusted according to these recovery values; range of recoveries for T: 53–70% and DHT: 50–69%. Average detection limits (as determined by water blanks) were 0.19 ng for T and 0.28 ng for DHT. Samples were run in duplicate, and precision (i.e., intra-assay variation) was measured by coefficients of variation (CVs). Average CVs for T and DHT were 2.3% and 2.4%, respectively. Consistency across the three assays (i.e., interassay variation) was determined by calculating average CVs for the standards, which are samples with known concentrations of hormones and used as controls in the assay. Interassay variation for T was 4.5% and for DHT it was 7.6%.

Data Analyses

Two variables related to the response to novelty (i.e., latency to first approach and latency to contact the feeder) were reduced to a single composite neophobia score by principal component analysis (PC1 explained 86.5% of variance; eigenvalue: 1.73) as these variables were highly correlated (Pearson's $r = 0.73$, $p < 0.001$). Approach and contact latency measures were adjusted prior to the principal component analysis by subtracting the baseline latency measures assessed prior to the trials in the absence of the novel feeder. Persistence in manipulating the novel feeder was measured by the PC1 score (79.8%, eigenvalue: 1.6) of other two correlated variables (number of approaches and number of pecks in the first trial, Pearson's $r = 0.6$, $p = 0.015$). Neophobia and persistence scores were calculated from only the initial response to reflect behavior given to a novel stimulus. If, for example, an individual first contacted the feeder in the second trial, then the duration of the first trial was added to the latency value. The resulting scores were compared across treatment groups by one-way ANOVA or Kruskal–Wallis test when variables did not meet the assumptions of a parametric test. Problem-solving success was compared with Fisher's exact test. The effect of each androgen on the measures of neophobia and persistence in manipulating the novel feeder were explored by correlation analyses in a data set pooled from the three treatment groups. In all tests, significance levels were set to 0.05. All tests were conducted using SPSS 22.0.

Results

Testosterone implants raised plasma levels of androgen significantly over both Blank-castrates and Shams (T mean ± SD: T-castrate: 7.37 ± 2.64 ng/ml, Blank-castrate: 0.37 ± 0.27 ng/ml, Sham: 0.57 ± 0.77 ng/ml, $F_{2,16} = 36.7$, $p < 0.001$; DHT mean ± SD: T-castrate: 1.72 ± 0.7 ng/ml, Blank-castrate: 0.12 ± 0.03 ng/ml, Sham: 0.08 ± 0.01 ng/ml, $F_{2,16} = 31.7$, $p < 0.01$), venturing into the range of free-living birds during breeding (T_{range} = 1.5–14.8 ng/ml, DHT_{range} = 0.3–2 ng/ml) (Wingfield & Farner 1978; Ramenofsky and Németh, in prep.). T-castrates were no more successful at problem solving than birds in the other groups (Fisher's exact test, $p = 1.0$). In fact, only one T-castrate was able to solve the feeding task and none from the other groups. Neophobia scores did not significantly differ across treatment groups (Kruskal–Wallis test, $\chi^2 = 3.54$, $p = 0.17$, Fig. 1); however, T-castrates showed more persistence in manipulating the novel feeder ($F_{2,13} = 6.8$, $p = 0.01$) than the Shams (Tukey HSD, $p = 0.01$) or the Blank-castrates (Tukey HSD, $p = 0.05$) (Fig. 2). Both T and DHT levels showed significant positive correlation with persistence scores (T: Spearman's $r = 0.595$, $p = 0.015$; DHT: Spearman's $r = 0.664$, $p = 0.005$; Fig. 3).

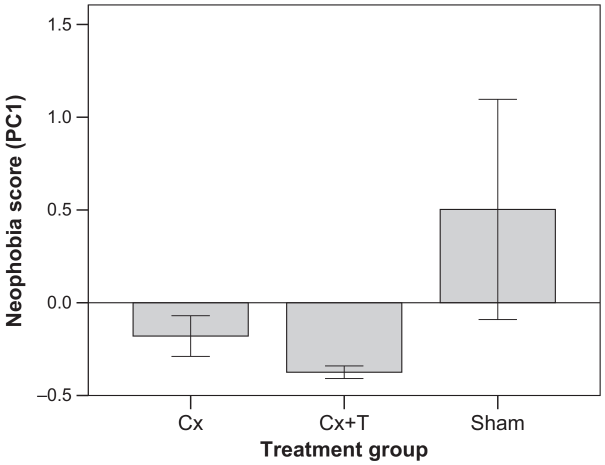

Fig. 1: Treatment groups (Cx: Blank-castrates, Cx+T: T-castrates, Sham: sham-operated controls) did not differ significantly in their neophobic response to the novel feeder. Neophobia (PC1) score is a PCA composite of two correlated variables: latency to approach and latency to contact the novel feeder. Negative score indicates less neophobic behavior.

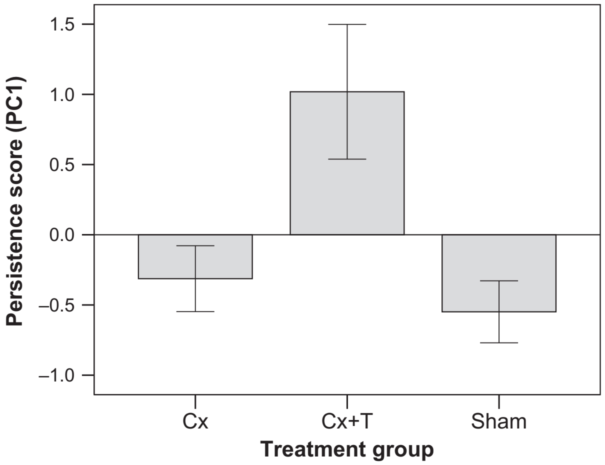

Fig. 2: T-castrates (Cx+T) were more persistent in manipulating the novel feeder than birds in the Blank-castrate (Cx) and sham-operated control (Sham) groups. Persistence (PC1) score is a PCA composite of two correlated variables: number of approaches and number of pecks in the first trial.

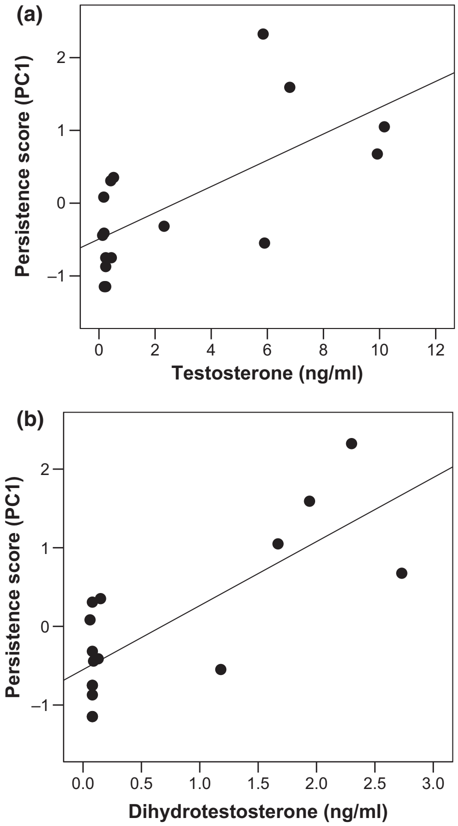

Fig. 3: Persistence in manipulating the novel feeder showed positive correlation with both T (a) and 5α-DHT (b).

Discussion

Gonadal steroids have been shown to play a role in modulating spatial learning and memory formation in mammals as well as birds (Frye et al. 2004; Oberlander et al. 2004; Hodgson et al. 2008). Their role, however, in other cognitive behaviors such as problem solving is still not well understood. In this study, we found no support for the hypothesis that androgens facilitate problem-solving behavior. On the one hand, we demonstrate a positive relationship of circulating levels of androgen (T and DHT) and persistence in manipulating a novel feeder during a problem-solving task in a songbird (Fig. 3). However, two of our other predictions were not supported. T-implanted birds were no more successful in problem solving than Blank-castrates or Shams, neither did they differ significantly in their neophobic response toward the novel feeder (Fig. 1). Given that only one bird was able to solve the novel feeder problem, we cannot conclude that androgens do not influence problem-solving ability. It is still plausible that in longer trials or on a different test, the effect of hormone treatment would be measurable.

Persistence in responding to a given stimulus has been suggested to be androgen dependent (Rogers 1974). For example, testosterone treatment increased persistence of food-searching behavior in both young and adult male domestic fowl (*Gallus gallus domesticus*), whereas antiandrogen treatment reversed it (Andrew 1972; Andrew & Rogers 1972; Rogers 1974). Our results confirm this pattern in songbirds and extend it by including a relationship of DHT that was also elevated with the T implant.

The androgen treatment in our study induced a breeding-like phenotype with increased cloacal protuberance size and pectoral muscle mass (Ramenofsky & Németh 2014). The corresponding increase in persistence in T-castrates suggests that this trait shows seasonal plasticity and may be part of the overall breeding phenotype. Increased persistence in response to a stimulus in the breeding season can be adaptive as the payoff for successful resource acquisition (e.g., territory, mate, food) is particularly high, at least in males (Lack 1968; Wingfield 1984). Consequently, one could speculate that birds with higher androgen titers in the breeding season may invest more effort in attempting to exploit novel resources and opportunities, which may become available at an increased rate in a rapidly changing global environment. If more persistent individuals are better at acquiring novel resources, we may predict that individuals with higher androgen production will more likely take advantage of, or survive in, rapidly changing landscapes.

Avoidance or fear of novelty (i.e., neophobia) is also suggested to be influenced by gonadal hormone levels in mammals (Cornwell-Jones & Kovanic 1981). For example, novelty-seeking behavior was reduced by suppressing gonadal hormone levels in rats (Cyrenne & Brown 2011). By contrast, the approach taken in many avian studies has mainly focused on the long-term effects of maternal steroids in behavioral responses to novelty. In pied flycatchers (*Ficedula hypoleuca*), androgen administration to eggs induced sex-dependent changes in adult exploratory and antipredator behavior but did not affect neophobic responses to novel objects (Ruuskanen & Laaksonen 2010). Similarly, experimentally elevated yolk androgens did not change the initial neophobic response to novel food in zebra finches (*Taeniopygia guttata*) (Tobler & Sandell 2007). Finally, short-term androgen treatment also failed to modify exploratory behavior, another cognitive behavioral trait, in a novel environment in house sparrows (*Passer domesticus*) (Mutzel et al. 2011). The lack of difference in neophobia across treatment groups in our study is consistent with the above-mentioned studies and suggests a less important role for androgens in mediating responses to novelty in birds than in mammals.

Conclusions

Understanding the hormonal regulation of adaptive behaviors is important as endocrine mechanisms can limit an organism's potential responses to changing environmental conditions (McGlothlin & Ketterson 2008; Wingfield 2013). To our knowledge, this is the first study to investigate the short-term effects of experimentally increased androgen on neophobia and problem-solving performance in an avian model. Our study demonstrates a link between circulating levels of androgens and persistence, but not neophobia, on a problem-solving task in males. A study using females may reveal different relationships. Although, we did not detect significant differences in problem-solving performance among treatment groups in two trials (i.e., total of 2 h), an experiment with longer exposure to a novel foraging task may yield different results if increased persistence leads to problem-solving success over time. Also, it is important to note that the type of test may influence how successful an individual is in innovative problem-solving; thus, multiple, ecologically relevant tests are recommended for future studies.

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