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Yolk testosterone modulates persistence of neophobic responses in adult zebra finches, *Taeniopygia guttata*

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Abstract

Individual differences in animal behavior can be attributed to genetic as well as non-genetic influences. One mechanism by which the behavioral phenotype of an individual can be shaped is via transmission of maternal sex steroids. In this study, we examined the role of yolk testosterone (T) in controlling neophobia in 9-month-old, sexually mature zebra finches (*Taeniopygia guttata*). Offspring hatched from either T-treated or control eggs were subjected to a sequential series of behavioral tests in which we measured the neophobic response and its persistence towards two unfamiliar stimuli. Birds from T-treated and control eggs did not differ in their latencies to approach and eat a novel food source during their first encounter. However, egg treatment affected subsequent habituation. Latencies decreased in both groups over a habituation period of 5 days, but considerably more so in T-offspring. Although males appeared to approach novel food faster than females, there was no overall sex effect during the habituation period. When a novel object was added in combination with the previously learned food stimulus, this caused an behavioral shift in approach latencies. In males, control offspring had significantly shorter latencies than T-offspring, whereas there was no difference among females. The latency to eat in the same test was not significantly affected by sex or egg treatment. Our results demonstrate long-term effects of prenatal T on neophobic responses in adult zebra finches. We hypothesize that prenatal T may be one underlying mechanism for individual differences routine formation.

Keywords: Maternal effects; Behavioral development; Testosterone; Neophobia

Introduction

In many animal species including humans, behavioral phenotype often varies individually (e.g. Boissy, 1995; Gosling, 2001; Sih et al., 2004). Identifying the underlying mechanisms for inter-individual differences in behavior is of crucial importance for the understanding of how variation in individual behavior is maintained within animal populations. Individual differences in behavioral patterns in many animal species are known to be under the control of genetic factors (Øverli et al., 2002; Drent et al., 2003; van Oers et al., 2004; Fairbanks et al., 2004; Dingemanse and Réale, 2005). However, there is increasing evidence that the development and expression of individual behavior is also strongly affected by non-genetic, maternal influences (e.g. Clark and Galef, 1995; Forstmeier et al., 2004; van Oers et al., 2004; Crews and Groothuis, 2005). One mechanism through which mothers can influence offspring behavior is maternal programming through hormones. Exposure to maternal steroid hormones during embryonic development is well known to have organizing effects on brain and behavior in many vertebrates (Clark and Galef, 1995; Collaer and Hines, 1995; Rhen and Crews, 2002). In birds, yolk androgens have been shown to influence boldness and competitive behavior of chicks (Eising and Groothuis, 2003; Daisley et al., 2005; von Engelhardt et al., 2006) as well as social dominance and sexual behavior in adults (Strasser and Schwabl, 2004; Eising et al., 2006). However, the organizational effects of egg steroids on adult behavior are still not very well known. Given that hormones often act on various target tissues (e.g. Ketterson and Nolan, 1999), they have the potential to modify the organization of a wide array of different behaviors. One important aspect is the role of androgens in controlling the persistence of specific behavioral patterns. Studies on poultry show that intramuscular administration of T in young chicken increases their persistence of

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attention to particular stimuli but reduces their ability to adjust to a new situation (e.g. Andrew and Rogers, 1972; Rogers, 1974; Andrew, 1975). We hypothesized that yolk androgens could have a similar organizational effect on persistence behavioral patterns and the ability to shift attention in adult birds.

In this study, we therefore examined long-term effects of yolk testosterone (T) on neophobia and its persistence in adult zebra finches (*Taeniopygia guttata*). Previous work on this species has shown that there is considerable variation in the amount of androgens deposited within and among clutches (Gil et al., 1999; Rutstein et al., 2005; Gilbert et al., 2005). Differential androgen transfer with respect to mate attractiveness or diet (Gil et al., 1999; Rutstein et al., 2005; Sandell et al., 2007) and sex-specific effects of yolk androgens on offspring begging and growth (von Engelhardt et al., 2006) suggest strategic investment and hence maternal programming in zebra finches. Neophobia, i.e. the fearfulness towards novel situations or stimuli, influences behavioral flexibility and has therefore been suggested to be an important factor affecting the ability to colonize new areas (e.g. Greenberg, 1990; Martin and Fitzgerald, 2005). Moreover, individual differences in neophobia are likely to translate into differences between behavioral phenotypes since neophobia is known to be correlated with other behaviors such as dominance or learning (e.g. Verbeek et al., 1996; van Oers et al., 2003; Boogert et al., 2006).

We experimentally manipulated yolk hormone levels in zebra finch eggs by *in ovo* injection of either T dissolved in sesame oil (T-eggs) or sesame oil only (control eggs). Offspring hatched from either T-or control eggs were subjected to a sequential series of behavioral tests in which we measured the reaction towards novel food, habituation to novel food and reaction to the a novel object when introduced after habituation. Sequential testing allowed us to compare learning effects between the treatment groups. Introduction of a novel object after some period of habituation enabled us to study how a learned behavioral pattern would change in case of a disturbance.

Methods

Animals and housing

Birds subjected to egg treatment and behavioral tests (see below) were bred in the laboratory facilities at Lund University, Sweden. The birds were maintained under constant light (14 L:10 D cycle) and temperature conditions (20± 2°C). Commercial finch seed, cuttlebone and water were provided *at libitum*. Juveniles were removed from their natal cages at 45–55 days of age and housed unisexually in cages (80 × 40 × 80) with 4–5 birds per cage. Individuals assigned to the same cage were matched with respect to sex and egg treatment (see below). All birds had acoustic and visual contact with adults of both sexes.

Testosterone injections

When egg-laying started, nests were checked daily and freshly laid eggs were replaced with artificial ones. Collected eggs were kept in an incubator (37°) until day 4 of incubation. On day 3, they were injected with either 500 pg testosterone (T) in 5 μl of sterile sesame oil (T eggs) or 5 μl sterile sesame oil only (control eggs). All eggs within a clutch received the same treatment and clutches were randomly assigned to treatment groups. We used the egg injection protocol previously employed by von Engelhardt et al. (2006) on this species. The hole in the egg shell was sealed with a tiny drop of superglue (Super Attak; Loctite Sweden AB, Göteborg, Sweden). After injection, eggs were immediately placed back into the incubator. On day four, eggs with live embryos were placed in foster nests. To uncouple parenting ability and egg quality, whole clutches were cross-fostered. Overall, the frequency of hatching failures in this experiment was not significantly different compared to the frequency of hatching failures in a breeding round in which eggs were not manipulated (manipulated: 27%, non-manipulated: 24%; $\chi^2=0.17$, $df=1$, $p=0.68$). The frequency of hatching failures, which were not due to infertility of the eggs, did also not differ significantly between treatment groups (T-broods: 14%, control broods: 25%; $\chi^2=1.69$, $df=1$, $p=0.19$). There was also no significant difference in brood size at hatching ($F_{1,23}=1.86$, $p=0.19$; T-broods: 3.0±0.2 chicks (mean±1 SE); C-broods: 2.5±0.3 chicks) and fledging ($F_{1,23}=1.45$, $p=0.24$; T-broods: 2.1±0.2 chicks; C-broods: 1.7±0.3 chicks).

Behavioral tests

Behavioral tests were based on 21 male (11 T and 10 control) and 23 female (17 T and 6 control) zebra finches from 25 families. Birds were tested when they were 8–10 months old, i.e. sexually mature. For practical reasons, males were tested at a slightly younger age than females (males: 261±3 days (mean±1 SE); females: 285±4 days). The exact age did not differ between treatment groups (egg treatment: $F_{1,21.7}=1.76$, $p=0.20$; sex: $F_{1,25..5}=62.13$, $p<0.05$; egg treatment × sex interaction: $F_{1,25.5}=0.05$, $p=0.82$). During behavioral tests, birds were housed individually in cages of 80 × 40 × 80 cm. Each cage had a standard set-up with one

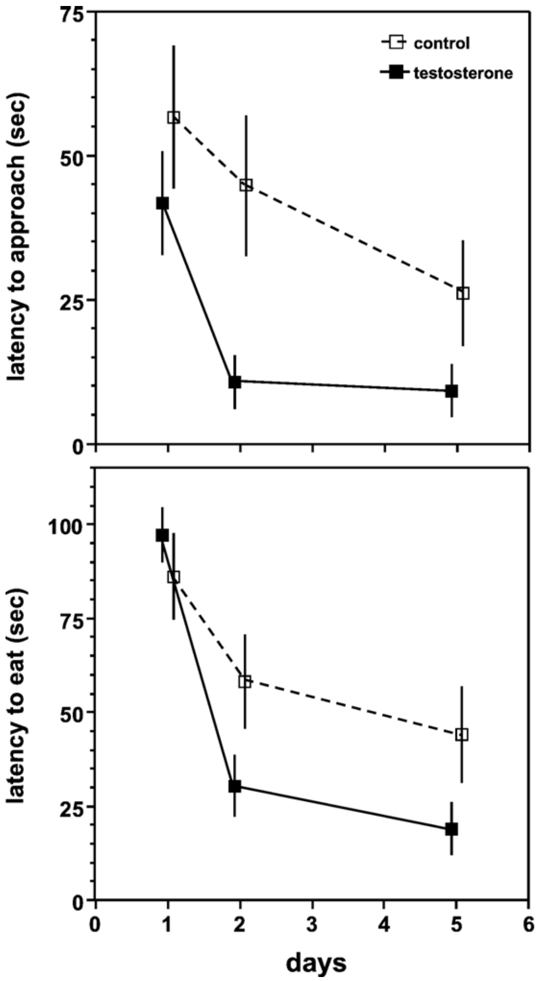

Fig. 1. Habituation towards novel food over a period of 5 days for ca. 9-month-old zebra finches hatched from testosterone-treated (filled squares, solid line) and control eggs (open squares, broken line). Data shown represent means (±1 SE) for the latency to approach and the latency to eat. Sample sizes: control: male=10, female=6; testosterone: male=11, female=17.