

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. © 2007 Elsevier Inc. All rights reserved.

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 *ad 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

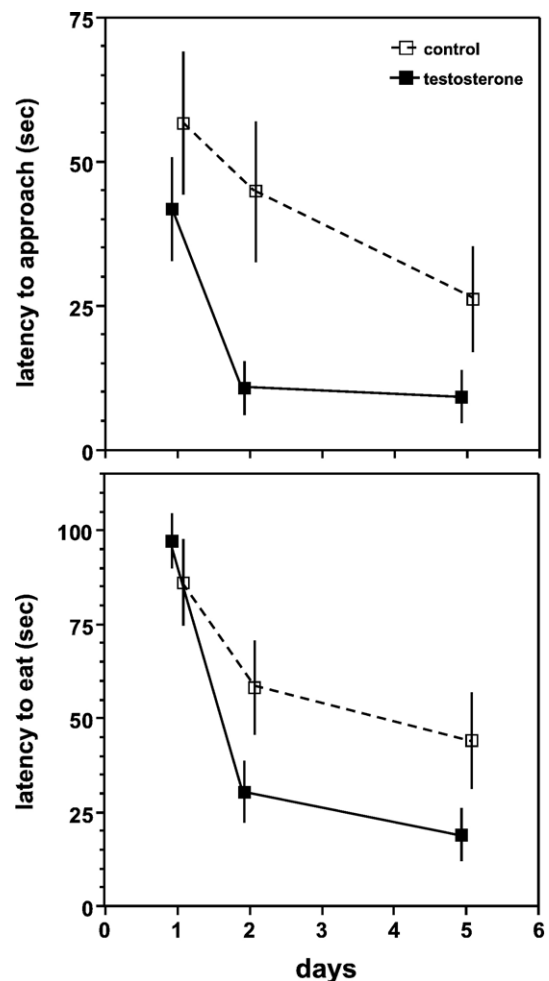


Fig. 1. Habituation towards novel food over a period of 5 days for ca. 9-month-old zebra finches hatched from testosterone-treated (filled squares, solid line) and control eggs (open squares, broken line). Data shown represent means (±1 SE) for the latency to approach and the latency to eat. Sample sizes: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

cross perch and two 15-cm side perches, seed mixture and water *ad libitum*. Birds had acoustic but no visual contact to other individuals during the experiment. To measure the birds' reaction towards novel food or a novel object, we used a similar protocol as described in Verbeek et al. (1994). Birds were transferred to the test cages, which were in a separate room, 1 day before the start of the experiment. We considered 1 day as sufficient for the birds to familiarize themselves with the test cage as these cages were identical to the ones the birds had been housed in until the start of the experiment. One day after the birds had been transferred to the test cage, the birds were given an approximately 4 × 4 cm piece of iceberg lettuce (the novel food), which was introduced on one of the side perches (trial 1). The birds had been raised and kept on a seed diet with no previous experience of lettuce or any similar food source. To test whether habituation would mitigate or enhance any behavioral differences due to egg treatment, birds were also given lettuce after 2 (trial 2) and 5 (trial 3) days (Fig. 1). On day 8 in the individual cage (trial 4), we performed a disturbance test in which the birds were again given lettuce, but this time in combination with a novel object (two toy plastic dinosaurs with black eyespots painted on them, Fig. 2). To avoid any side bias, we introduced the lettuce and the novel object on the side perch opposite to the one used in the preceding three trials. All tests were conducted between 08.30 and 14.00 h. As the light was switched on at 07.45, this means that birds had at least 45 min during which they could eat and drink undisturbed before the onset of the first test. We filmed the birds during the first 120 s after the introduction of the lettuce/novel object with a video-camera (Panasonic®, Type MS4) and later scored their behavior on a screen. The behavior was quantified using two measures: (i) the latency time for the bird to sit on the perch with the lettuce/the novel object (latency to approach) and (ii) the latency

time to start eating the lettuce (latency to eat). Birds that did not approach or eat during the trial received a maximum time (120 s). Each individual was involved in four trials spread over 8 days, resulting in 176 (4 × 44) trials. However, on 2 occasions the camera did not work properly, and hence we only obtained 174 recordings. The person scoring the behavior was blind with respect to the egg treatment of the birds.

Data analysis

Statistical tests were conducted with SAS System for Windows, Version 9.1 (SAS Institute, 2004). Behavioural data were ranked (PROC RANK) to normalize the residuals. We used mixed model repeated measures ANOVA (PROC MIXED; Littell et al., 2004) with zebra finch identity as the subject to analyze the change in neophobic responses over the first 3 trials. Egg treatment, sex and experiment trial were modeled as fixed factors. We used autoregressive covariance structure of order one, which yielded the model with the lowest Akaike criterion (Littell et al., 2004). The identity of the biological mother as well as brood nested within foster pair identity was initially included as random factors in the model to account for the fact that some females produced several clutches and that some foster pairs raised more than one brood. The behavioral response in trial 4 was analyzed with linear mixed models, using egg treatment and sex as fixed factors, latency time of trial 3 as a covariate and the identity of the biological mother and brood nested within foster pair identity as random factors. The covariate was included to control for individual differences in motivation to approach and eat the lettuce. All interactions between fixed factors and covariates were initially included, but non-significant effects ($p > 0.1$) were sequentially removed from the models. Random effects were estimated with the likelihood ratio test (Littell et al., 2004). In trial 4, the effect of the biological mother on approach latency could not be estimated as any model including this effect failed to converge. Therefore, only the effect of the foster environment could be estimated in this case. However, although the identity of the biological mother did explain a significant part of the variation in few of the full models testing for behavioral differences, exclusion of this factor did not affect the results qualitatively in any of the models. It is therefore unlikely that the results would be different in the model in which it was omitted. Foster environment had no significant effect in any of the models ($\chi^2 < 1.7$, $df = 1$, $p > 0.10$). As models without this factor gave qualitatively the same results, it was omitted from all models. In a few specific cases, e.g. when testing the association between behavioral responses in trials 1 and 4, normality of the residuals could not be achieved with the ranking procedure. We therefore used Spearman rank correlation to estimate the association. The denominator degrees of freedom were calculated using the Satterthwaite approximation (Littell et al., 2004). The likelihood ratio test for random factors was one tailed. All other tests were two tailed. The significance level was set at $p < 0.05$.

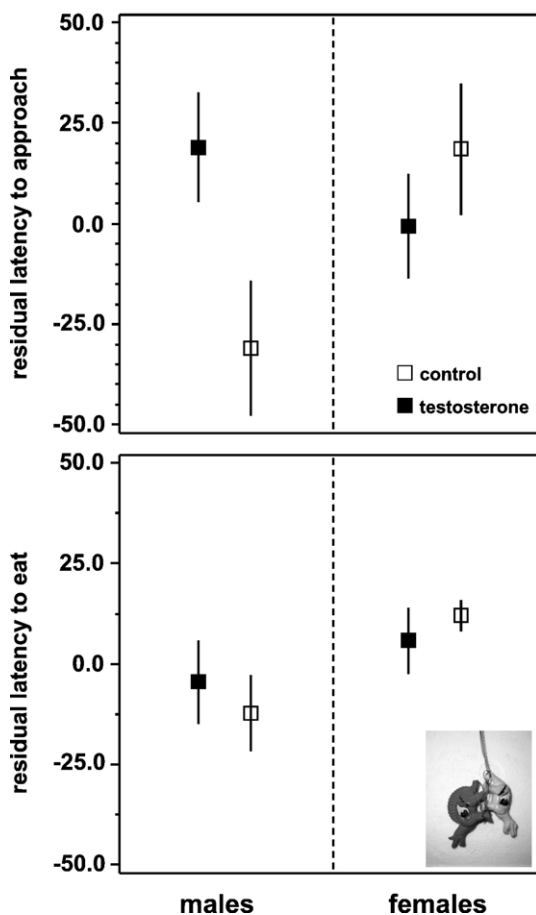


Fig. 2. Behavioral response towards a novel object, presented after habituation to novel food for ca. 9-month-old zebra finches hatched from testosterone-treated (filled squares) and control eggs (open squares). Data shown represent residuals from the statistical model (Table 2) controlling for latencies to approach and eat in the preceding test (trial 3), but without including egg treatment and sex as a factor. The picture in the lower, right corner shows the novel object. Sample sizes: control: male=10, female=6; testosterone: male=11, female=17.

Results

Response to novel food and habituation

Body mass at the beginning of the experiment did not differ between treatment groups, but females weighed slightly more than males (egg treatment, $F_{1,35.7} = 0.61$, $p = 0.44$; sex, $F_{1,32.8} = 5.09$, $p = 0.031$; egg treatment × sex interaction, $F_{1,28.9} = 0.34$, $p = 0.56$). Seventy-eight percent of the variation in individual body mass was explained by the effect of sharing the same biological mother ($\chi^2 = 14.7$, $df = 1$, $p < 0.001$).

In the first trial, the latency to approach did not differ significantly between T- and control offspring ($F_{1,39.6} = 2.53$, $p = 0.12$), although T-offspring were somewhat faster to approach than C-offspring (T: 38 ± 10 s (mean ± 1 SE); control: 56 ± 13 s). There was no significant interaction between sex and egg treatment for the first trial ($F_{1,40} = 0.85$, $p = 0.36$), but males had significantly shorter approach latencies than females ($F_{1,41.7} = 4.40$, $p = 0.042$). Although the effect was significant for both T- and control offspring

combined, further analysis revealed that the overall effect can be attributed to sex differences among T-offspring (T: males 25 ± 15 s, females 51 ± 13 s, $F_{1,25.6} = 6.45$, $p = 0.018$; control: males 49 ± 16 s, females 62 ± 20 s, $F_{1,11.5} = 0.02$, $p = 0.90$). Latency to eat during the first trial did not differ significantly with respect to egg treatment or sex (egg treatment: $F_{1,42} = 1.30$, $p = 0.26$; sex: $F_{1,40.7} = 0.12$, $p = 0.89$; sex \times egg treatment: $F_{1,39.6} = 2.04$, $p = 0.16$).

Over the whole period of the first 5 days, T-offspring were faster to approach than C-offspring (Table 1) and this despite an apparent habituation effect in both treatment groups (Fig. 1). This overall effect was due to significantly shorter latencies of T-offspring in trials 2 and 3 ($F_{1,40} = 10.93$, $p = 0.002$; $F_{1,37.5} = 5.91$, $p = 0.02$). There was significant inter-individual variation in the latencies to approach ($\chi^2 = 17.1$, $df = 1$, $p < 0.0001$). Individual identity explained 33% of the variation in approach latencies. Latency to eat also decreased over the first three trials in both treatment groups, but more dramatically in T-offspring than in control offspring resulting in a significant interaction between trial number and egg treatment (Table 1, Fig. 1). Overall, T-offspring started eating faster than C-offspring during the first 5 days of the experiment, but this effect was due differences in latencies in trials 2 and 3 ($F_{1,40} = 4.59$, $p = 0.038$; $F_{1,28.6} = 5.15$, $p = 0.031$; Fig. 1). Latency to eat was significantly different between individuals ($\chi^2 = 15.6$, $df = 1$, $p < 0.0001$). Inter-individual variation explained 43% of the total variation in latency to eat.

Disturbance after habituation

Introduction of the novel object into the test cage together with the lettuce increased both latency to approach and latency to eat. The latency to approach in trial 4 was significantly positively related to the approach latency in trial 3 (Table 2). There was a significant interaction between egg treatment and sex for the latency time to approach in trial 4 (Table 2, Fig. 2). This was because control males were faster to approach compared to T-males ($F_{1,18} = 9.12$, $p = 0.007$), whereas there was no differ-

Table 1
Effects of sex, egg treatment and trial number on the behavioral response towards novel food (lettuce) during the first three trials

Factors	df	F/ χ^2 value	p-value
<i>(a) Latency to approach</i>			
Sex	1,41.7	0.63	0.43
Egg treatment	1,35.2	8.3	0.007
Trial	2,78.8	19.57	<0.001
Sex \times trial	2,78.8	2.76	0.07
Biological mother	1	0.7	0.20
<i>(b) Latency to eat</i>			
Sex	1,22.9	0.82	0.38
Egg treatment	1,16.8	4.56	0.048
Trial	2,78.3	36.05	<0.001
Trial \times egg treatment	2,78.3	4.42	0.015
Biological mother	1	1.3	0.13

Presented is the reduced model. Non-significant ($p > 0.1$) main effects and their interactions were backward eliminated from the full model. The identity of the genetic mother was included as random factors in the model. For fixed effects, the test statistic is F ; for random effects, it is χ^2 .

Table 2

The effect of sex and egg treatment on the behavioral response towards the novel object in trial 4

Factors	df	F/ χ^2 value	p-value
<i>(a) Latency to approach</i>			
Sex	1,39	0.87	0.36
Egg treatment	1,39	1.96	0.11
Sex \times egg treatment	1,39	5.31	0.028
Trial 3 latency	1,39	8.37	0.006
Biological mother		*	
<i>(b) Latency to eat</i>			
Egg treatment	1,39.9	4.54	0.039
Trial 3 latency	1,39.9	3.33	0.076
Egg treatment \times trial 3 latency	1,39.6	3.68	0.062
Biological mother	1	0.2	0.33

*Not tested (see Methods).

The latency time in trial 3 was included as a covariate to control for differences in motivation to approach and eat. Presented is the reduced model. Non-significant ($p > 0.1$) main effects and their interactions were backward eliminated from the full model. The identity of the biological mother was included as random factor in the model. For fixed effects, the test statistic is F ; for random effects, it is χ^2 .

ence between control and T-females ($F_{1,15.5} = 0.44$, $p = 0.52$). The latency to eat in the presence of a novel object, on the other hand, was not significantly affected by sex (Table 2), although the pattern was similar to the one for approach latency (Fig. 2). There was, however, a significant effect of egg treatment affecting the relationship between the latencies in trials 3 and 4 (Table 2). Behavioral responses between trials 3 and 4 tended to be correlated among control offspring (Spearman rank correlation, $r_{16} = 0.46$, $p = 0.074$), but not among T-offspring ($r_{28} = -0.0004$, $p = 0.99$).

Neither the latency to approach nor the latency to eat during the first trial (first exposure to novel food) was significantly related to the latencies in trial 4 (novel object) (Spearman rank correlation, $r_{44} < 0.18$, $p > 0.25$ in both cases). This was also true if the behavioral correlation between trial 1 and trial 4 was analyzed separately for each treatment group (Spearman rank correlation, $r < 0.4$, $df > 15$, $p > 0.13$).

Discussion

Our study demonstrates long-lasting effects of prenatal T on the neophobic responses of an altricial passerine, the zebra finch. Egg treatment did not affect the initial response to a novel stimulus but instead affected learning. Even though we cannot distinguish whether birds reacted to the novel food or the experimental procedure per se, it is apparent that offspring hatched from T-treated eggs habituated more quickly than offspring from control eggs. One possible explanation for these results may be that egg T treatment had an organizing effect on behavior and neural function in our zebra finches. Organizational effects occur during a short critical period early in the development and create permanent changes in the phenotype (e.g. Arnold and Breedlove, 1985). They stand in contrast to activational effects, which alter the behavioral state temporarily and are, thus, reversible (e.g. Arnold and Breedlove, 1985). Long-lasting effects of prenatal

androgen exposure may be caused by differences in sensitivity to these androgens in adulthood or by permanent changes of hormone-secretion patterns resulting in higher circulating levels of androgens later in life. A classical example for organizational actions of hormones during early development is the differentiation of alternative behavioral phenotypes (territorial versus non-territorial morphs) in male tree lizards (*Urosaurus ornatus*; Moore, 1991; Moore et al., 1998). Interestingly, the effects of prenatal T elevation found in our study are similar to activational effects of T found in chickens. T injected in young chickens induces a more persistent attention to a stimulus (e.g. Andrew and Rogers, 1972; Andrew, 1975; Clifton et al., 1988). As a consequence, behavioral patterns are more likely to reassert themselves once they have been initiated (Andrew and Rogers, 1972; Rogers, 1974; Andrew, 1975; Archer, 1976; Clifton et al., 1988). At the same time, T injection delays the shift towards a new stimulus since it stabilizes the use of earlier learned specifications and hence reduces the ability to adjust to a new situation (Clifton et al., 1988). It is suggestive, that prenatal egg T may have an organizing effect on androgen production later in life causing an attention-stabilizing effect on behavioral patterns comparable to the above mentioned effect of in vivo administered T in poultry. Hence, T-offspring may be able to respond more quickly to a known stimulus (i.e. lettuce) once it has been established, which would result in shorter latencies. However, adult hormone levels were not measured in the present study, and thus we do not know whether T-offspring had consistently higher levels of circulating androgens.

Possibly, prenatal manipulation of T could also have affected sexual differentiation in our zebra finches. As we determined the sex of the birds on the basis of the sexually dimorphic adult plumage, we cannot exclude that egg treatment had a demasculinizing or feminizing effect on the brain and behavior of male zebra finches. Demasculinization may occur if the injected T was aromatized to estrogen, which is known to play a major role in avian sex differentiation (Balthazart and Adkins-Regan, 2002). Hence, sex-specific treatment effects such as found in trial 4 with the novel object could be interpreted as feminization of males by prenatal T. It is interesting to note that the effect of administered testosterone increasing the persistence of attention to a particular stimulus in chickens seems to occur only in males (Andrew and Rogers, 1972; Andrew, 1975).

However, although the concept of early organizational effects of maternal hormones is appealing, it has recently been suggested that long-term effects of maternal hormones may also be indirect and not organizational (Carere and Balthazart, 2007). Behavioral differences in adulthood may be due to differences in early social interactions mediated by early activational effects of prenatal hormones. As pointed out by Carere and Balthazart (2007), social behavior in adulthood may be determined by social status experienced early in life. A recent study by von Engelhardt et al. (2006) found sex-specific effects of yolk T on nestling begging behavior and growth in zebra finches. Female offspring hatched from T-eggs begged more compared females from control eggs, whereas no difference in begging was found for males. As the effects of prenatal T on begging disappeared after 1 week post-hatch, it has been suggested that the effect of egg T may be transient activational one (Carere and Balthazart, 2007). It is

possible that the behavioral differences among adult zebra finches in our study is due to differences in early competitive environment (i.e. begging intensity) mediated by maternal androgens.

An alternative explanation for differences in latencies to approach and eat between T- and control offspring may be a difference in the motivation to eat. A recent study has demonstrated that elevation of prenatal T results in an increase of nestling metabolic rate in the zebra finch (Tobler et al., 2007). If this difference in metabolism would persist into adulthood, it could be argued that increased metabolic rate in T-offspring could translate into higher hunger levels. However, we think that this is a less likely explanation. Birds had *ad libitum* access to seeds throughout the experiment and behavioral tests never started directly after the light was switched on in the morning. Birds had at least 45 min during which they could eat undisturbed before they were tested.

Studies on birds and other vertebrates have shown that that boldness or neophobia is correlated with a suite of other behaviors such as aggressiveness, exploratory behavior, sociability and activity (Huntingford, 1976; Verbeek et al., 1996; van Oers et al., 2003; Kazlauckas et al., 2005; Boogert et al., 2006). It is thus likely that differences in learning due to egg treatment are associated with differences in other behaviors. Forstmeier et al. (2004) found strong maternal effects on the sexual behavior (male aggressiveness, male song rate, female mate preference) of adult zebra finches. Hence, assuming such a correlation between neophobic responses and social behavior in zebra finches, it is likely that the maternal effects found by Forstmeier et al. (2004) are, at least partly, mediated by maternal hormones. Further work is needed, however, to determine the relative importance of maternal hormones with respect to other non-genetic influences on offspring behavioral phenotype.

Differences in the persistence of neophobic responses are likely to have important fitness consequences, especially if they translate into differences in other behaviors (see above). It is possible that the effect of yolk testosterone on neophobia in zebra finches is associated with fitness benefits. Behavioral flexibility in terms of higher propensity to utilize unfamiliar food sources may influence individual ability to colonize new areas (e.g. Greenberg, 1990; Martin and Fitzgerald, 2005). However, a reduction in neophobia can also be interpreted as an increase in risk taking. Thus, a reduction in neophobia may not only entail benefits but also costs. Costs and benefits of neophobic responses are likely to depend on the social context or the environment (e.g. Verbeek et al., 1994; Dingemans et al., 2004; Dingemans and Réale, 2005).

In summary, our study shows that high levels of egg androgens can have long-term effects on neophobic responses. Variation in prenatal androgen exposure may explain individual differences in individual differences in routine formation.

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