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The hormonal control of begging and early aggressive behavior: Experiments in black-headed gull chicks

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Abstract

The hormonal control of begging and sibling competition is largely unknown, but recent evidence suggests a role for steroid hormones. We tested the influence of the aromatizable androgen testosterone (T), the non-aromatizable androgen 5α-dihydrotestosterone (DHT), and 17β-estradiol (E) on both begging behavior and aggressive behavior in black-headed gull chicks (*Larus ridibundus*). Chicks of this species have a conspicuous begging display, while their frequently performed early aggressive behavior is facilitated by testosterone and important for territorial defense. Hormone treatment was applied by implants between days 6 and 16 after hatching. Behavior was tested by means of standard stimulus tests. The results were validated in a second experiment under semi-natural conditions. Begging was suppressed by T and DHT and not affected by E. Aggressive Pecking was strongly facilitated by T. The erect threat posture, characteristic for older chicks, was facilitated by T, DHT, and E and the nest-oriented threat display, typical for young chicks, only by T and DHT. Growth was suppressed in the T group. The results indicate that androgen production, needed for territorial defense, has costs in terms of a suppression of begging and growth. It is discussed to what extent older chicks may avoid these costs by converting testosterone to estrogen and why pre-natal and post-natal exposure to androgens differ in their effect on begging behavior.

Keywords: Testosterone; Androgens; Estrogen; Begging; Development; Aggression; Sibling competition

Introduction

Much is known about the role of gonadal steroids on the organization and activation of adult social behavior. However, despite the fact that early social behavior can be of great importance for survival (Mock and Parker, 1997), not much is known about the influence of gonadal hormones on social behavior early in ontogeny. In avian species, early treatment with testosterone can induce precocial social display and copulatory behavior (for a review, see Groothuis, 1993), but the function of gonadal hormones in the regulation of social behavior of young birds under natural condition is still largely unexplored. Recently, the hormonal basis of sibling competition has attracted some attention, but the data do not yet show a coherent picture. In white storks (*Ciconia ciconia*), first hatched chicks are more aggressive, receive more food, and have higher plasma levels of testosterone than their siblings. The number of younger siblings that die is higher when the difference in testosterone levels between first and later hatched chicks is greater (Sasvári et al., 1999). Although the data are not based on experimental evidence, they suggest a role for testosterone in sibling competition and/or begging behavior. In the blue-footed booby (*Sula nebouxii*), a facultative siblicidal species, dominant chicks did not show higher testosterone levels than subdominant chicks (Nuñez-de la Mora et al., 1996; Ramos-Fernandez et al., 2000). However, in the closely related Nazca booby (*Sula granti*), plasma levels of testosterone, but not those of DHEA and cortisol, were elevated immediately after actual fights between siblings

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(Ferree et al., 2004). Finally, Groothuis and Meeuwissen (1992) reported that testosterone treatment of black-headed gull chicks (*Larus ridibundus*) strongly facilitated territorial defense but inhibited the begging display. Although they did not quantify the latter, it is to our knowledge the only published experimental study on the influence of post-natal exposure to androgens on a begging display in a bird species.

The possible suppressing effect of testosterone on begging behavior is, however, not supported by two other studies. Androgen injections into the yolk of eggs before incubation induced a higher frequency of begging behavior after hatching, not only in chicks of the canary (*Serinus canaria*, Schwabl, 1993), but also in the black-headed gull (Eising and Groothuis, 2003). This contradiction suggests that pre-hatching and post-hatching treatment with testosterone have different effects on begging behavior.

A suppressive effect of post-natal testosterone exposure on begging behavior would make the case of the black-headed gull chick especially interesting. These chicks frequently perform both conspicuous begging displays and aggressive behavior, including threat displays. In this colonial breeding species, chicks defend small territories around the nest and young chicks produce testosterone contingent on the level of aggressive challenges (Ros et al., 2002). Furthermore, within individuals, these testosterone levels correlate with the frequency of aggressive behaviors, while testosterone treatment strongly facilitates aggressive behavior (Groothuis and Meeuwissen, 1992; Ros et al., 2002). Together, the available information suggests that the gull chicks face the problem that androgens, required for territorial defense, at the same time inhibit begging behavior, which is needed for obtaining food from the parents. In that case, the suppression of begging is a possible cost of producing testosterone that is needed for territorial defense, in addition to other costs of the hormone such as reduced growth (Ros, 1999), the development of a less cryptic plumage (Ros, 1999), and malformation of the syrinx (Groothuis and Meeuwissen, 1992). To reduce these costs, territorial behavior becomes increasingly sensitive to relatively low levels of androgens in the course of the first 4 weeks after hatching (Groothuis and Meeuwissen, 1992; Ros et al., 2002). One mechanism underlying this increase in sensitivity and avoiding the costs of testosterone exposure might be to increase aromatase activity that converts testosterone into estrogen. This hormone has been shown to facilitate aggressive behavior in many species, including adult laughing gulls (*Larus atricilla*), a closely related species of the black-headed gull (Terkel et al., 1976), while it might not influence the syrinx or suppress growth and the frequency of begging behavior.

In order to test the hormonal background of both begging and aggressive behavior, we implanted chicks of the black-headed gull with testosterone, estrogen, or 5α-dihydrotestosterone, a non-aromatizable androgen. Controls received sham surgery. The effect of treatment on begging, aggressive behavior, and growth was assessed in standard tests in hand-reared birds (experiment 1). In addition, the effect of testosterone and estrogen on begging and aggressive behavior was validated under semi-natural conditions (experiment 2, in a breeding colony housed in a large aviary). Based on the available literature of black-headed gulls, we expected that the androgens would suppress begging behavior and growth and stimulate aggressive behavior. In addition, we tested whether estrogen would stimulate aggressive behavior typical for older chicks while it would, in contrast to the androgens, not suppress begging behavior and growth.

Materials and methods

Experiment 1: hormonal specificity of social behaviors in standard tests

Rearing conditions

Black-headed gull chicks of 2–3 days of age were collected in June from a large gull colony in the north of The Netherlands and hand reared at the laboratory where they were housed in groups of three or four peers. Groups were held in adjacent cages measuring 85 × 75 × 85 cm and could not see each other. The floors of the cages were covered with straw. Each cage contained a thermal lamp providing constant dimmed light and a temperature of approximately 37°C in the middle of the cage. Additional tube lighting had a 16L:8D schedule. The chicks were individually marked (on the head or back) with rhodamine or picrine (ICN Biochemicals, Cleveland, Ohio; chemicals were dissolved in acetone). Food and water were available ad libitum. During the first 2 weeks, chicks were fed with a moistened mixture of pellets used in trout farming (Trouvit, Trouw, Gent) and a mixture used for growing chicks (Sivo start, Bogena, Waalwijk). This basic diet was supplemented daily with smelt (*Osmerus eperlanus*) and mashed hard-boiled chicken eggs. At 2 weeks of age, the diet gradually shifted to dry trout pellets, with egg added twice a week. A vitamin supplement (Calviet, UTD, Meppel) was added weekly.

Design

Forty-six animals were housed in ten groups of four animals and two groups of three animals. Groothuis and Meeuwissen (1992) showed that such groups are excellent for conducting hormonal studies. Endogenous testosterone production of chicks reared in such groups is retarded due to the lack of social stimulation by conspecifics from other groups. Such chicks behave towards each other as they normally do in sibling groups, showing no aggression and threat display behavior while frequently give begging displays. At 6 days post-hatching in every group, chicks were assigned randomly to one of the four treatments: no hormonal treatment: C: $n = 11$; testosterone implants: T: $n =$

12; 17β-estradiol implants: E: $n = 11$; and 5α-dihydrotestosterone implants: DHT: $n = 12$. One T-bird escaped from the cage and died after the last behavioral test but before morphometrical measurements could be taken (see below). One T-bird, one DHT-bird, and one control bird died within the first 3 weeks after hatching, and all their data were removed from the data set. Ten days after implantation, hormone treatment was ended by removal of the implant. Standard behavioral tests (for details, see below) were carried out once every day during the second half of the treatment period (treatment days 7 to 10). Effectiveness of the implants was checked by determining steroid levels in blood collected 7 days after start of treatment in about half of the number of birds. To assess the effect of treatments on growth, body weight was measured at the start and end of the implantation period. At the latter time point, the length of head and bill was taken as a measurement of skeletal growth. All experiments were carried out under specific licenses of the Dutch government and the university ethical committee for animal experiments and adhere to equivalent of the NIH guidelines.

Hormonal implants

Hormones were purchased from Diosynth, Oss, The Netherlands. Since a pilot study revealed that E and DHT diffused much less readily from silicon tubes than T, all hormones were melted into crystalline pellets of 25 mg. Pellets were implanted subcutaneously in the neck region under local anesthesia with lidocaine (Xylocaine, Astra, Rijswijk, The Netherlands). The incision was closed with stitches. Control birds received sham surgery. Similar implants but with testosterone propionate were shown in an earlier study to induce moderate levels of T (1.45 ng/ml; Groothuis and Meeuwissen, 1992), within the physiological range of birds in social groups (1–2 ng/ml, Ros et al., 2002).

Effect of sex

Sex was determined by measuring the head + bill length at the age of 2.5 months. In fledged birds, older than 5 weeks, this measure is in ±95% of the cases smaller than or equal to 8.1 cm in females and larger than 8.1 cm in males (Coulson et al., 1983; Koopman, 1990). Since some birds had died before the age of 5 weeks, we could not determine sex of all birds (except from the four birds mentioned above, none of them had died during or within a week after the experiment). Furthermore, since in the T-group, growth of head and bill was significantly reduced, we could not reliably determine sex in this group. The number of reliably sexed males and females in the treatment groups was as follows: C: 4 and 3 resp.; DHT: 5 and 5 resp.; E: 6 and 4 resp. Based on these data and the fact that chicks had been randomly allocated over the groups, sex is very unlikely to be a confounding factor in our analyses. Furthermore, within each treatment group, the frequencies of the different types of behavior did not differ between the sexes. Moreover, in some cases, we were able to obtain larger sample sizes for testing sex effects by taking several treatment groups together since different treatments had a similar effect on behavior. In these cases with a larger statistical power, we still found no indication for sex-specific effects of the hormones. This is not surprising since the species is monomorphic in both plumage and most behaviors (van Rhijn, 1985), and both in this species and the closely related laughing gull both sexes react similarly to androgen and estrogen treatment (Groothuis and Meeuwissen, 1992; Terkel et al., 1976).

Blood sampling and radioimmunoassay

Seven days after implantation, 0.5 ml blood was drawn from the brachial wing vein with a heparin-rinsed needle and syringe within 5 min of capturing the bird. After centrifugation, plasma was stored at 20°C. Radioimmunoassays were carried out at the Department of Herd Health and Reproduction at the University of Utrecht. Steroids were extracted from the plasma samples with diethyl ether. DHT levels were estimated on the basis of the cross reactivity of the antibody for T with DHT (see below). For methods and evaluation of the radioimmunoassays used, see Dieleman et al. (1983) for testosterone, and Dieleman and Bevers (1987) for 17β-estradiol.

Previous studies at the Utrecht laboratory showed that, in the assay for estimating testosterone levels, the main levels of cross-reactivity with the antiserum were 49.7%, 7.54%, and 3.35% for 5α-dihydrotestosterone, 4-androstene-3β,17β-diol, and androstenedione, respectively. In our assays, lower detection levels of 0.05 ng/ml T and upper detection levels of 4 ng/ml T were used. The main cross-reactivities of the antisera used in the assay for 17β-estradiol were 1.1%, 0.32%, and 0.16% for estrone, estriol, and 17α-estradiol, respectively, and <0.01% for other steroids tested (according to the manufacturer of the antisera, Coat-A-Count TKE; Diagnostic Products Corporation). In our assays, lower detection levels of 5 pg/ml E and upper detection levels 400 pg/ml E were used. Analyses were done within a single assay to avoid inter-assay variability, and intra-assay variation was 5%.

Hormone levels

After the experiment was finished, the birds were used for a study on the influence of hormones on immunocom-

Table 1
Effect of 10-day hormone treatment on steroid plasma levels

Treatment	Estradiol (ng/ml)	Androgen (ng/ml)
C	$0.018^{a} \pm 0.004$ ($n = 10$)	$0.06^{a} \pm 0.02$ ($n = 10$)
DHT	$0.047^{a} \pm 0.016$ ($n = 5$)	$0.92^{b} \pm 0.29$ ($n = 4$)
E	$0.269^{b} \pm 0.046$ ($n = 5$)	$0.18^{a} \pm 0.12$ ($n = 5$)
T	$0.055^{a} \pm 0.021$ ($n = 5$)	$1.58^{b} \pm 0.43$ ($n = 5$)

Different letters refer to significant differences between groups, tested with the Newman–Keuls post-hoc test.

petence. These data have been published already together with the results of the radioimmunoassays (Ros et al., 1997). These hormone data are summarized in Table 1 (statistics based on ANOVAs and Newman–Keuls post-hoc tests). Since we did not separate DHT and T by chromatography and the antibody we used for both T and DHT had an almost 50% cross reactivity for DHT, we refer to total androgens in the table. As expected, the T-treatment elevated the androgen level in the blood plasma to the upper part of the physiological range (Malickiene, 1999; Ros et al., 2002). Based on the cross reactivity of the antibody with DHT (about 50%), the actual circulating DHT level in the DHT group will have been around that of T in the T-treated birds.

Behavioral tests

The effect of hormone treatment on begging behavior and aggressive behavior was measured in standard stimulus tests at day 7, 8, 9, and 10 after the start of treatment.

Begging test. We hand-reared the chicks by offering them food on the tip of an outstretched finger, simulating the parent's bill. Chicks quickly started to beg upon the introduction of a finger in their cage. During the begging tests, chicks were offered food by hand as during normal feeding for 3 min.

Begging in this species consists of two behaviors (Groothuis, 1989a): (1) a conspicuous pumping display in which head and neck move rapidly up and down in the vertical plane. During each downward movement, a loud short lasting call is uttered. This is predominantly performed when the chick sees and approaches the parents from a distance; (2) when close to the parent, a specific high pitch short lasting begging call is uttered, the so-called pee call, either during pumping or with the head drawn in against the body. Both behaviors are often performed in long bouts. We recorded whether a chick had performed either pumping and or pee calls during the 3 min, regardless of its frequency, or not at all.

Aggression test. This test took place about 10 min after the begging test. The chicks were confronted for 5 min with a stuffed adult conspecific in their home cage, simulating an adult intruder in the territory, and their behavior was recorded on videotape. This test was developed for young black-headed gulls. The model was mounted on a long stick and held for 2 min in front of the cage, 2 min in the middle of the cage, and 1 min close to the birds that would normally withdrawn in a corner of the cage (for details, see Ros et al., 2002).

Aggressive behavior was classified on the basis of the position of head, body, neck, and wings and on movements and accompanying calls of the bird, according to Groothuis (1989b): (1) All erect postures accompanied by loud calls were classified as the Oblique. In chicks, this display has, compared with other displays, the highest temporal association with Aggressive Pecking. (2) All postures in which the head was held in front of the body while the bill was not pointing downwards were classified as Forward. Chicks use these postures during begging or submission. (3) All postures in which the bill was pointed downward and which were accompanied by repetitive calls were taken together as Choking. Chicks use Choking frequently at the nest site during aggressive interactions; (4) each separate aggressive peck was classified as Aggressive Pecking.

Sexual behavior, including head-flagging (facing away) and copulation (attempts), did not occur.

Statistical analysis

The difference in begging behavior between the different treatment groups was tested as follows. The percentage of tests in which a bird performed begging was calculated for each bird over days 7, 8, 9, and 10 after start of treatment. This is the period, based on our previous experiments (e.g. Groothuis and Meeuwissen, 1992; Ros et al., 2002), in which the effect of treatment on behavior was expected to be maximal. These percentages were arcsine transformed and tested by means of ANOVA and Newman–Keuls tests.

To test differences in aggressive behavior between the experimental groups, a MANOVA was carried out, with as dependent variable the mean frequency of each type of aggressive behavior over the same period as for begging followed by univariate statistics and Newman–Keuls post-hoc tests, to test the effects of separate treatments and separate behaviors. Frequencies of behavior were Poisson transformed (Zar, 1984).

Differences in growth (in terms of changes in body mass), and skeleton measurements were tested with an ANOVA and Newman–Keuls post-hoc tests.

Alpha was set to 0.05, and we only refer to statistical significant effects when $P < 0.05$.

Experiment 2: semi natural-context

Rearing condition

We used chicks that hatched from nests of our breeding colony at the laboratory. The colony consisted of 30 second-year and adult birds, of which five pairs laid and incubated eggs. They were housed in an aviary of 15 × 6 m, which contained a large pond and several food containers. Food consisted of pellets for trout farming (Trouvit, Trouw, Gent) and was ad libitum available. Near the nests with chicks at least once a day, moistened food was provided as in experiment 1. All birds had color bands for individual recognition by the observer. In addition, chicks were colored as in experiment 1.

Design, treatment, and observations

We used broods of four nests, two containing initially 3 chicks, and two containing two chicks. One chick of a three-brood nest left its nest within a few days after hatching and was adopted by the parents of the other three-chick nest. This created three nests of two and one nest of four chicks.

At day 6 after hatching, one chick in each nest was implanted with a pellet of crystalline testosterone (T). In the three nests containing two chicks, the other received sham surgery (C). In the remaining nest of four chicks, two received sham surgery and one was implanted with a pellet of 17β-estradiol. Implants and way of treatment were similar as in experiment 1.

Begging and aggressive behavior of the chicks was observed and recorded with the help of a tape recorder, from a hide attached to the middle of the long side of the aviary. We observed 1.5 h per day during the same period as in experiment 1. Classification of behavior was also similar as in experiment 1, except for begging. Since the pee call could not always be heard, we scored only pumping bouts during which the pee call is often performed.

Statistical analyses

We used two sample *t* tests on the Poisson transformed data of pumping and the Poisson transformed data of the total number of aggressive behaviors (Oblique, Choking, and Aggressive Pecking).

Results

Experiment 1: hormonal specificity of social behaviors

Begging

Treatment significantly affected the incidence of begging behavior (Fig. 1; ANOVA: $F(3,39) = 8.93$, $P < 0.001$). The Newman–Keuls tests revealed that T and DHT caused a significant decrease in begging behavior compared to that of the control group and the E group (Fig. 1). Begging did not differ significantly between the E group and the C group.

Aggressive behavior. The overall MANOVA showed a strong effect of hormonal treatment on behavior (Wilks' lambda (12,95.54) = 0.352, $P < 0.001$). Univariate tests were significant for Aggressive Pecking (Fig. 2A, $F(3,39) = 3.03$, $P = 0.041$), Oblique display (Fig. 2B, $F(3,39) = 10.88$, $P < 0.001$), and Choking display (Fig. 2C, $F(3,39) = 5.73$, $P = 0.002$). Forward was performed in low frequency and not significantly different between groups (Fig. 2D, $F(3,39) = 0.395$, n.s.).

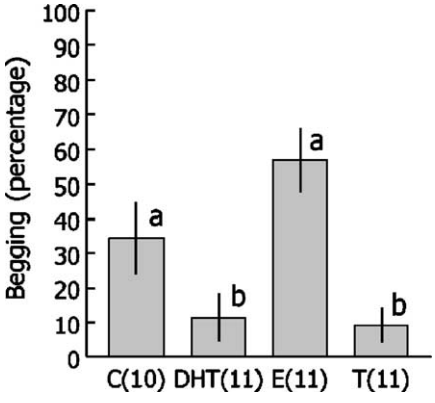

Fig. 1. Mean percentage (±SEM) of begging behavior in four subsequent daily begging tests by black-headed gull chicks in relation to hormone treatment: C: controls, sham surgery; DHT: 5α-dihydrotestosterone; E: 17β-estradiol; T: testosterone. Sample sizes between brackets. Different letters refer to significant differences between groups, tested with the Newman–Keuls post-hoc test.

According to the Newman–Keuls tests, testosterone treatment significantly increased the frequency of both Aggression Pecking and the Oblique and Choking displays. DHT treatment had similar effects on aggressive behavior as the T-treatment but its effect on Aggressive Pecking did not reach statistical significance.

The effect of E treatment on Aggressive Pecking did not differ from that of T and DHT, but its enhancing effect was not strong enough to show a statistically significant difference with the control group (Fig. 2A). E treatment was as effective as T treatment in elevating the frequency of Oblique display and tended to be more effective than the non-aromatizable DHT, but this latter effect did not reach statistical significance (Fig. 2B). Interestingly, the effect of E-treatment on Choking clearly differed from that of T and DHT treatment (Fig. 2C). Only the androgens but not estrogen stimulated this nest site related posture.

Growth

The increase in body weight over the implantation period (Fig. 3A) was affected by treatment (ANOVA with treatment as factor (4 levels): $F(3,38) = 3.36$, $P < 0.05$). Post-hoc analyses showed, as expected, that T treatment significantly decreased growth rate compared to control treatment. E and DHT treatment did not affect growth rate and were significantly different from T-treatment. No significant correlation was found between the total frequency of all aggressive behaviors and the increase in body weight (Pearson correlation, $r < 0.20$, n.s.).

Head + bill length (Fig. 3B) was clearly affected by treatment too (ANOVA with treatment as factor (4 levels): $F(3,38) = 8.40$, $P < 0.001$). Again, this was due to smaller head + bill lengths in the T-treated chicks than in the other groups. E and DHT did not affect skeleton size when compared to the control group.

Experiment 2: semi-natural condition

Begging

Like in the previous experiment, T-treated birds performed less begging than controls (mean ± SEM in frequency per hour: T-group: 1.1 ± 0.4 h^{-1}; C-group: 5.8 ± 1.4 h^{-1}; two-sample *t* test $t(4,5) = 4.73$, $P < 0.01$). The E-treated chick had the highest frequency of begging of all chicks (12.3 h^{-1}).

Aggressive behavior

Again like in experiment 1, aggressive behavior, now calculated as the total frequency of Oblique, Choking, and

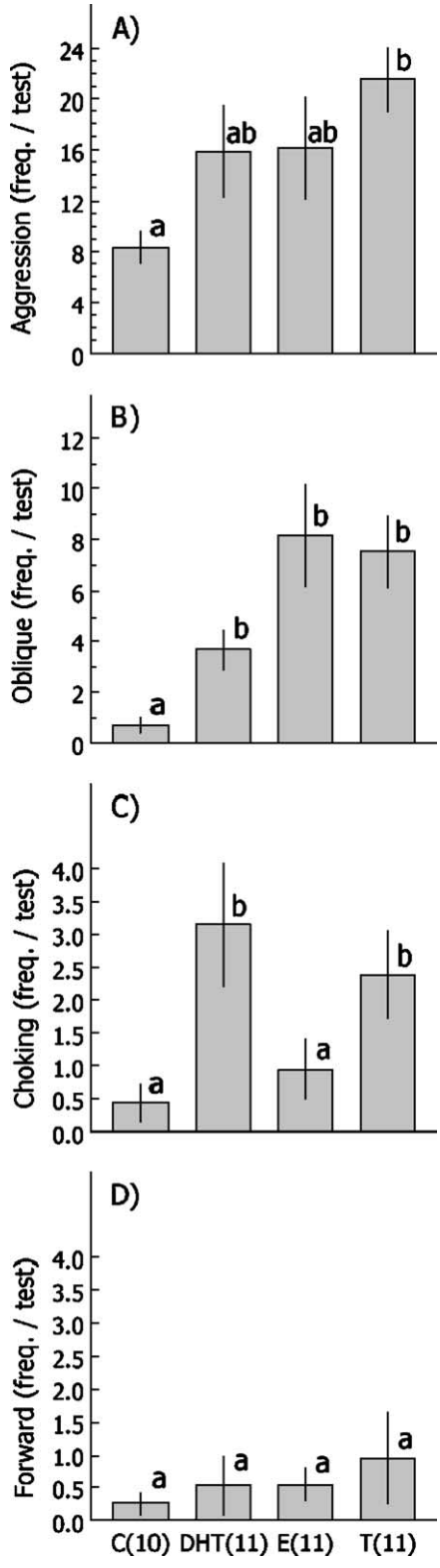

Fig. 2. Mean frequency (±SEM) of aggressive behavior over four subsequent daily standard stimulus tests (5 min) by black-headed gull chicks in relation to hormone treatment. (A) Aggressive Pecking; (B) Erect Oblique display; (C) Nest-oriented Choking display; (D) Forward display. Different letters refer to significant differences between groups, tested with the Newman–Keuls post-hoc test. Sample sizes between brackets.

Aggressive Pecking, was much higher in the T-treated than in the Control chicks (T: 9.56 ± 2.18 h^{-1}; C: 1.15 ± 0.11 h^{-1}, two sample t test: $t(4,5) = 4.73$, $P < 0.01$).

Discussion

The main aim of this paper is to determine the hormonal control of begging behavior and aggressive behavior in chicks of a species for which both behaviors are important early in ontogeny and for which conflicting evidence for this hormonal control has been reported, while testosterone has been demonstrated to inflict some costs on the growing chick (see Introduction). To the best of our knowledge, our results present the first quantitative and experimental evidence, both under laboratory and semi-natural conditions, that begging behavior is suppressed by androgens, supporting the impression by Groothuis and Meeuwissen (1992). Aggressive behavior is strongly facilitated by androgens that at the same time suppress growth, consistent with the literature for this species (Groothuis and Meeuwissen, 1992; Ros, 1999; Ros et al., 2002). Like this early between brood competition, early within brood competition in the form of aggression is likely to be facilitated by testosterone (Ferree et al., 2004). This indicates that the two forms of sibling competition, in the form of aggression and begging respectively, differ in their hormonal control.

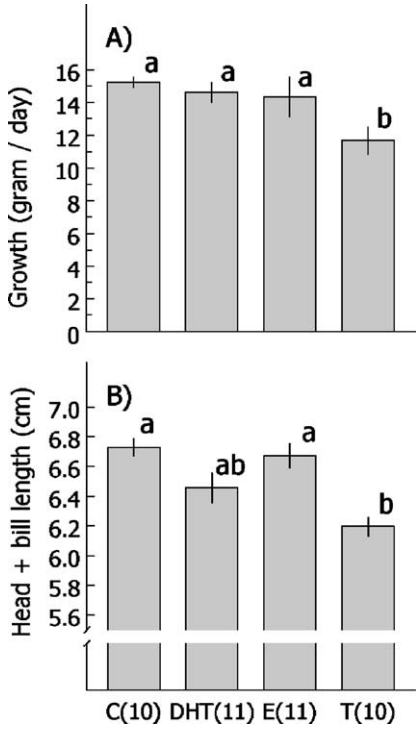

Fig. 3. Growth (mean ± SEM) in black-headed gull chicks in relation to hormone treatment. (A) Increase in body weight over the implantation period. (B) Size of head + bill at the end of the implantation period. Different letters refer to significant differences between groups, tested with the Newman–Keuls post-hoc test. Sample sizes between brackets.

The suppression of begging and growth by androgens may represent important costs for the chick. Begging facilitates the parents to regurgitate food (Eising and Groothuis, 2003), and the chick is completely dependent on this food source for its survival. Growth retardation may lead to later fledging, at a stage that many breeding pairs already leave the colony, weakening the defense against predators. However, elevated levels of androgens are likely to be important for effective territorial defense by means of aggressive behavior (Ros et al., 2002). Interestingly, and in line with our expectation (see last paragraph of the Introduction), estrogen facilitates part of the aggressive repertoire while it does not suppress begging and growth. This provides the chicks with the possibility to perform part of the aggressive repertoire without the inhibition of begging and growth.

However, estrogen, stimulating the Oblique display, did not facilitate the Choking display. The Choking display is characteristic for chicks of less than 2 weeks old and decreases in frequency in older young in which the Oblique display is the predominant display (Groothuis, 1989a). The finding that Choking is facilitated by DHT but not by E while E strongly facilitates the Oblique suggests that the production of estrogen may increase only some weeks after hatching. This is understandable for two reasons: first, because in birds, early exposure to estrogen can affect sexual differentiation (Balthazart and Adkins-Regan, 2002). Second, because the defensive Choking display, during which the young bird crouches in the vegetation, may be especially important for small and vulnerable young chicks, while the offensive and conspicuous Oblique display is especially of importance in an older stage. The difference in hormonal control between the two displays may provide the chick a mechanism for the required independent control of both behaviors. To our knowledge, the only evidence for differences in hormonal control between different displays of the same species has been found in the ring-dove (*Streptopelia risoria*, Hutchison and Steimer, 1984; Fusani et al., 2001).

The increase in estrogen production during the first weeks after hatching, as suggested above, would be consistent with our earlier study on the hormonal control of aggressive behavior in this species. We have shown elsewhere that in the course of the first weeks after hatching young gulls increase the sensitivity of social behavior to androgens and suggested that this may be a strategy to avoid the costs of androgens on growth and begging behavior (Groothuis and Meeuwissen, 1992; Ros et al., 2002). We now suggest that an increase in aromatase activity may be part of this mechanism, perhaps due to early testosterone production itself since testosterone has been shown to up-regulate aromatase activity (Balthazart, 1997; Balthazart et al., 1990, 1994; Steimer and Hutchison, 1981).

While our post-hatching treatment of testosterone suppressed begging strongly, pre-hatching treatment with T or T and androstenedione has been shown to increase begging, both in the black-headed gull (Eising and Groothuis, 2003) and in the canary (Schwabl, 1993). The results suggest age-dependent effects of testosterone on begging, as has been found for sexual behavior in the domestic chicken (*Gallus gallus domesticus*, Clifton and Andrew, 1989). Experimental evidence indicates that pre-hatching treatment increases the mass of the neck muscle used in begging (m. complexus, Lipar and Ketterson, 2000). Furthermore, the nucleus supraspinalus as well as the syrinx, both an integrated part of the motor control system of begging (Schwabl and Lipar, 2002), contain androgen receptors (Gahr et al., 1996). Interestingly, a transient expression of androgen receptors has been found in several parts of the motor system for begging, occurring in the embryo but disappearing early after hatching, suggesting an organizational role for androgens on begging behavior (Godsave et al., 2002). So, it is conceivable that yolk androgens mainly organize the motor apparatus for begging while post-hatching exposure to these androgens influences central–motivational aspects. Perhaps elevated levels of post-natal androgens shift priorities in the bird from foraging and maintenance to territorial behavior. Correlational and experimental studies suggest that corticosterone may activate begging behavior (Kitayski et al., 2001; Schwabl and Lipar, 2002). Androgens, known for their effects on growth of neural and muscular tissue, may then positively affect the motor system for begging pre-natally, while glucocorticostroids, known to regulate energy housekeeping, positively affect the motivation to beg post-natally.

Growth

Growth in terms of body weight and skeleton size was, even in the ad libitum food conditions, suppressed in T but not in E-treated chicks while DHT showed a non-significant tendency to suppress skeleton growth too. The effect of T is in agreement with the results of an earlier study with this species (Ros, 1999) and with fowl (Deyhim et al., 1992; Fennell and Scanes, 1992; Wise and Ranaweera, 1981). This finding might be explained by several not mutually exclusive possibilities: first, by the costs of increased levels of activity due to androgen treatment. However, territorial behavior was also increased in E-treated chicks whereas growth rate was not decreased in these birds. Furthermore, we did not find a correlation between the total frequency of aggression (Aggressive Pecking, Choking, and Oblique) and growth. Second, it might be that androgens specifically influence thermal conductance by suppression of fat deposition as has been found in quail (Feuerbacher and Prinzinger, 1981) or by enhancing peripheral blood flow. Third, androgens may increase metabolic rate. This has been found in two studies (in a songbird, the house sparrow (*Passer domesticus*): Buchanan et al., 2001; in a cichlid fish: Ros et al., 2004) but not in another study (songbird, white-crowned sparrow (*Zonotrichia leucophrys*: Wikelsky et al.,

1999). Such an increase in metabolic rate may be due to the building up of relatively costly muscle tissue or immune tissue. Indeed, it was found in the birds of this specific experiment that DHT and T, but not E, increase immunocompetence (Ros et al., 1997); finally, androgens may suppress feeding behavior.

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