Roles of Sex Steroids in the Crowings with Sexual and Non-Sexual Motivations in Female Japanese Quail, *Coturnix japonica*

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Received March 22, 2013; revised April 23, 2013; accepted May 2, 2013

ABSTRACT

To examine the sex differences in the crowing behavior of the Japanese quail, we investigated the effects of sex steroids on calling behaviors using female birds, and the data were compared with those obtained in our previous study in male birds which was performed using the same experimental procedures as those in the present study. We injected the female quails daily from 11 to 41 days after hatching with testosterone propionate (TP), 5α-dihydrotestosterone (DHT; a non-aromatizable androgen), estradiol benzoate (EB) or vehicle, and examined their calling behaviors in both sexual and non-sexual contexts. In a non-sexual context of the birds being isolated in a recording chamber, androgens, either TP or DHT, induced crowing in place of distress calling while EB simply inhibited distress calling. These effects of sex steroids on the calling behaviors were almost identical to those in the male quails. In a sexual context of the birds being left undisturbed in their home cages, crowing was induced by chronic treatment with TP, but not either DHT or EB, suggesting that both estrogenic and androgenic actions are required to induce the sexually motivated crowing. Although these results were basically the same as those in the male quails, the crowing in the female quails occurred much less frequently compared to that of the male quails. These data suggest that in the Japanese quail, crowing behavior, when it is restricted to sexually motivated one, is quantitatively different between male and female in the responsiveness to sex steroids.

Keywords: Japanese Quail; Crowing; Distress Call; Androgen; Estrogen

1. Introduction

In male Japanese quails (*Coturnix japonica*), testosterone (T) activates the entire sequence of copulatory behaviors, including grabbing the neck of the female, mounting and achieving cloacal contact. In female quails, activating effects of T are different from those in males. Ovariectomized females never show male copulatory behavior after treatment with amounts of T that are behaviorally effective in males [1]. Crowing, a courtship call, normally observed in sexually mature males, on the other hand, is not sexually differentiated in the organizational sense because T treatment readily elicits this behavior in ovariectomized females [2].

It is well known that male copulatory behavior is activated by T after being metabolized into both estrogenic and androgenic metabolites [3,4]. Crowing, on the other hand, is thought to be strictly androgen-dependent because either T or its non-aromatizable androgenic metabolite 5α-dihydrotestosterone (DHT) activates this behavior in adult gonadoctomized quails of both sexes [2, 4-7]. Moreover, even in chicks of both sexes, distress calling, which is induced when they are socially isolated, is replaced with crowing within a few days after subcutaneous implantation of either T or DHT [8]. This observation suggests that crowing can be induced by activation of androgen receptors (ARs) not only in a sexual context but also in a stress (non-sexual) context regardless of degree of sexual maturation.

Our recent study, performed by using male Japanese quails, however, suggested that activations of both ARs and estrogen receptors (ERs) are required to induce crowing as a courtship call (crowing with sexual motivation) while acute isolation stimulus can induce crowing (crowing with non-sexual motivation) if only ARs are activated [9]. In this study, the male quails were injected...
daily with either testosterone propionate (TP), DHT, estradiol benzoate (EB) or vehicle, starting on Day 11 (11 days after hatching) until Day 42. During this maturation period from chicks to adult birds, we observed their calling behaviors in both a recording chamber (acutely isolated conditions) and their home-cages (well-acclimated conditions). These procedures distinguished between the crowing with sexual motivation and that with non-sexual motivation because the former was mainly observed in their home-cages while the latter was observed only in the recording chamber. In these experiments, the birds treated with TP, but not DHT, began to crow in their home-cages much earlier than vehicle-treated controls, as a consequence of T-induced acceleration of sexual maturation. On the other hand, crowing in the recording chamber, which was occurring in place of distress calling under acutely isolated conditions, was observed after only a few days’ treatment with either TP or DHT.

We recently examined the effects of demasculinization by prenatal estrogen exposure on calling behaviors in male Japanese quails [10]. In this study, we applied the same procedures for posthatching treatments with sex steroid (TP, DHT or EB) and recordings of calling behaviors as those used in our previous study mentioned above [9]. The demasculinized male quails never crowed in their home-cages even after chronic TP treatment, while they crowed in the recording chamber after treatment with either DHT or TP as often as the birds which were treated prenatally with vehicle and then received corresponding posthatching hormonal treatment. These data suggested the possibility that crowing, when limited to sexually motivated one, is highly differentiated between males and females. It should be considered, however, that the prenatal estrogen exposure to male embryos does not necessarily mimic the in vivo effects of ovarian hormones in intact females [11,12].

The present study was designed to examine the sexual dimorphism in crowing behavior of Japanese quail by investigating the crowings with both sexual and non-sexual motivation in female quails. For the purpose of comparison, we again applied the same experimental procedures for hormonal treatments and recordings of calling behaviors as those used in our previous study in male quails [9].

2. Materials and Methods

Female Japanese quail chicks (Coturnix japonica) were purchased from a local breeder (Tokai Yuki, Aichi, Japan) one day (Day 1) after hatching and were kept in an incubator at 36°C in colonies of 10 - 20 chicks. From Day 5, they were housed in cages containing 3 - 4 chicks each, as described below. On Day 8, all of the cages were transferred to an air-conditioned room maintained at 28°C. Water and food were provided ad libitum. Lights were on for 14 h/day, between 900 and 2300.

The birds were randomly assigned to 5 experimental groups (7 - 9 birds/group) on Day 5, and housed in cages (home-cage; 35 cm long × 24 cm wide × 25 cm high) containing 3 - 4 birds from the same group. The birds in intact group (Group INT) were left without hormonal treatment throughout the experiments. The birds of the other four groups received for 31 days (Day 11 to 41) one of the following daily subcutaneous injections: testosterone propionate (TP; Wako, Osaka, Japan; 10 μg/g b.w., Group TP), 5α-dihydrotestosterone (DHT; Wako; 10 μg/g b.w., Group DHT), estradiol benzoate (EB; Wako; 1μg/g b.w., Group EB) or a vehicle (Group VEH). All injections were in 2 μl/g b.w. sesame oil containing 5% ethanol and were performed between 1200 and 1400. Since the body weight of the quails abruptly increases during the period of hormonal treatments (from Day 11 to 41), we chose to inject the quails daily with hormones rather than treating them with hormone-filled Silastic capsules. This allowed us to administer the same relative dose of hormones throughout the duration of the experiment.

For all experimental groups, calling behaviors induced by acute isolation were examined between 900 and 1200 every second or third day from Day 6 to Day 41. Acute isolation stimulus was given by isolating a bird in a test cage (30 cm long × 15 cm wide × 22 cm high) placed in a sound-proof recording chamber. Each bird was adapted to the test cage for 2 min, and then the numbers of distress call and/or crow were counted during next 3 min. These calls were monitored with a microphone in the recording chamber. It was easy to distinguish crowing from distress calls by acoustic and visual inspections because of their respective stereotypic acoustic patterns and postural displays. Each group and each bird within each group were observed in random order. During each session, the cloacal gland area (CGA) was measured with a caliper (largest width × largest length, in mm²), as this structure is androgen sensitive and its surface area provides a sensitive indicator of each birds’ hormonal condition [13].

The numbers of crows in home-cages were also counted on Days 26, 29, 33, 36, 39 and 41 between 1500 and 1800. In general, frequencies of crowing observed in sexually matured adult males are much lower than those of distress calling in chicks. In the present experiments, crowing in home-cages was promoted by exposing the birds to tape-recorded crows of conspecific males. The 6 min-long stimulus tape was edited so that it contained about 90 crows from two adult males, and was repeatedly played back during a 30-min test session. Three cages were observed simultaneously and the order of observation was changed daily.

During the experiments, we checked the conditions of
all birds every day. Some birds in groups TP, INT and VEH severely attacked other birds in the same home-cages. In these cases, we first changed the combination of the birds within the same group. However, if this procedure did not reduce the aggressive behavior, we transferred the dominant birds to individual home-cages and they were kept isolated thereafter. All data collections were continued for these isolated birds throughout the experiment. The numbers of calls in the recording chamber, however, were not used for later analyses because chronically isolated birds would not respond to an acute isolation stimulus upon placement in the recording chamber. One bird in Group TP which was badly wounded by other aggressive bird(s) and two birds in Group EB which were unhealthy probably due to the toxic effects of continuous estrogen treatment were removed from the experiments. For the remaining birds in each group, we adjusted the number of birds per cage to two or more so that they were not kept isolated until the end of the experiment. The numbers of both total and isolated birds in each group used for the behavioral and morphological data collections are shown in Table 1.

Differences in the percentages of the birds emitting calls among the groups were analyzed by two-tailed Fisher exact probability tests and differences in frequencies of calls were analyzed by the Kruskal-Wallis analysis of variance followed by two-tailed Mann-Whitney U-tests. CGA was analyzed by one-way analyses of variance (ANOVA) followed by Tukey tests.

The methods employed in this research adhere to the NIH standards described in the Guide for the Care and Use of Laboratory Animals (DHEW Publication 80 - 23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD).

3. Results

3.1. Distress Calling and Crowing in the Recording Chamber

In vehicle-treated controls (Group VEH) and intact birds (Group INT), the frequencies (number/3 min) of distress calling (Figure 1(a)) and the percentages of birds emitting this call (Figure 2(a)) gradually decreased as the birds grew older. Hormonal treatments markedly affected distress calling; comparisons of the frequencies of distress calling in the 5 groups (INT, VEH, TP, DHT and EB) by the Kruskal-Wallis test showed a significant difference from Day 15 till 29 \( \chi^2 = 25.758, P < 0.001 \) (Day 15); \( \chi^2 = 31.303, P < 0.001 \) (Day 18); \( \chi^2 = 29.553, P < 0.001 \) (Day 20); \( \chi^2 = 26.048, P < 0.001 \) (Day 22); \( \chi^2 = 23.480, P < 0.001 \) (Day 25); \( \chi^2 = 12.494, P < 0.05 \) (Day 27); \( \chi^2 = 13.866, P < 0.01 \) (Day 29)].

In Groups TP and DHT, both the frequencies of distress calling and the percentages of birds emitting this call sharply decreased within several days after the beginning of the hormonal treatments, showing the levels significantly lower than those in Groups VEH and INT. In its place, the birds began to crow (Figures 1(b) and 2(b)) from Day 13 and 15 in Groups TP and DHT, respectively. At first, the crowing occurred in a mixed manner with distress calling, but by Day 18, distress calling in both the TP-treated birds and the DHT-treated birds completely disappeared. In Group TP, however, crowing in the recording chamber (Figures 1(b) and 2(b)) was maintained at levels lower than those in Group DHT from Day 18 until the end of the experiments. The differences were significant on Days 20 and 25 for the frequency of crowing (Figure 1(b)) and on Day 20 for the percentage of birds crowing (Figure 2(b)).

In Group EB, distress calling strongly diminished within several days after the beginning of the hormonal treatments, showing the levels significantly lower than those in Groups VEH and INT (Figures 1(a) and 2(a)). Distress calling completely disappeared in 5 of 6 birds by Day 15. Although the remaining one bird emitted distress call until Day 29 and on Day 36, the calls of this bird sound somewhat different from normal ones. The birds from Group EB, as well as the birds from Groups INT and VEH, never crowed in the recording chamber.

### Table 1. Number of birds in each experimental group used for behavioral and morphological data collection during the experiments.

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The numbers of birds which were isolated in their individual home-cages by the day of data collection were shown in parentheses. Decrease in the number in Groups TP and EB means removal of severely wounded or obviously unhealthy birds from these groups.

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3.2. Crowing in the Home-Cages

Crowing in the home-cages was observed only in the birds from Group TP. Figures 3 and 4 show the frequency of the crowing and the cumulative percentage of birds crowing, respectively. One of the 7 TP-treated birds (14.29%) began to crow from Day 29, and 3 of the 7 birds (42.86%) crowed by Day 41. The birds from the other 4 groups (Groups INT, VEH, DHT and EB) never crowed in their home-cages throughout the present experiments.

3.3. CGA

As shown in Figure 5, treatments with the sex steroids strongly affected the CGA. Differences between groups were significant from Day 13 till the end of the experiment [ANOVA with \( P < 0.001 \) on each day; \( F(4,34) = 12.588 \) (Day 13), \( F(4,34) = 17.980 \) (Day 15), \( F(4,34) = 36.360 \) (Day 18), \( F(4,33) = 67.362 \) (Day 20), \( F(4,31) = 57.826 \) (Day 22), \( F(4,31) = 81.205 \) (Day 25), \( F(4,30) = 151.072 \) (Day 27), \( F(4,30) = 182.574 \) (Day 29), \( F(4,30) = 140.843 \) (Day 32), \( F(4,30) = 105.519 \) (Day 34), \( F(4,30) = 97.762 \) (Day 36), \( F(4,30) = 73.735 \) (Day 39), \( F(4,30) = 66.122 \) (Day 41)]. CGA of the birds from Groups TP, DHT and EB sharply increased after the onset of the hormonal treatments, and became significantly larger than that of the birds from Groups INT and VEH within a few days. The difference in the gland size among Groups TP, DHT and EB was also significant from Day 20 (Group DHT > Group TP > Group EB). In Groups INT and VEH, gland size increased only slightly until about Day 29, and then began to obviously increase to the level of Group EB. No significant difference among Groups INT, VEH and EB was observed after Day 39. In these three groups, however, the glands remained flat and never became bright red, in contrast to those of the androgen-treated birds from Groups TP and DHT. Instead, the glands showed estrogen dependent female-typical development; an enlargement of the cloacal diameter rather than a growth of the gland itself, indicating that estrogen released from their maturing ovaries was dramatically increased after about Day 29 in Groups INT and VEH.

4. Discussion

4.1. Crowing with Non-Sexual Motivation

In the present experiments, the treatment with either TP or DHT, but not EB, induced crowing in the recording chamber within several days after the onset of the treatment. The crowings occurred in place of distress callings which were induced by acute isolation stimulus. The
treatment with EB, on the other hand, sharply decreased distress calling without replacing it with crowing. These results suggest that androgens produce crow vocalization by acting on the vocal control system without affecting the motivational system underlying distress calling, while estrogens seem to exert inhibitory action on the motivational system without affecting the vocal control system. In the birds from Group TP, the levels of the crowing in the recording chamber remained lower than those in the birds from Group DHT after Day 15 (Figures 1(b) and 2(b)). This would be explained, at least in part, by the inhibitory action of T-derived estrogen on the motivational system for distress calling.

It is conceivable that the age-dependent decrease of distress calling in the birds from Groups INT and VEH is also involved in the inhibitory action of estrogen on the motivational system. In these birds, estrogen released from their ovaries would increase concomitant with their sexual maturation as indicated by estrogen dependent female-typical cloacal gland development (Figure 5). Indeed, distress calling in Groups INT and VEH completely disappeared by Day 39, when CGA in these groups reached a level almost equal to that observed in Group EB. In contrast to distress calling, crowing in the recording chamber in both Groups DHT and TP was observed until the end of the experiments (Day 41) (Figures 1(b) and 2(b)). For Group DHT, this may be explained by lower inhibitory action of estrogen on the motivational system for distress calling. In the birds from this group, estrogen released from their own ovaries would have remained low because of negative feedback of DHT on gonadotropin secretion [14]. For Group TP, on the other hand, acute isolation-induced crowing would have been decreased by inhibitory action of T-derived estrogen on the motivational system. Instead, the birds in this group probably began to crow with sexual motivational even in the recording chamber from about Day 29 when they began to crow in their home-cages (see Figures 3 and 4).

Using the same procedures as used in the present experiments, we previously examined the effects of sex steroids (TP, DHT or EB) on calling behaviors in male Japanese quails [9]. In this study, the frequencies of both distress calling and crowing in the recording chamber and the percentages of the birds emitting these calls showed almost the same age-related profiles as those in the female birds treated with corresponding sex steroid in the present study. Taken together, these studies suggest that the hormonal responsiveness of the neural substrates...
mediating the acute isolation stimulus-induced crowing is not sexually differentiated at the level of either the performance or the motivation. In both male and female quails, activation of ARs would be required to produce crow vocalization in place of distress call and the activation of ERs would exert inhibitory effects on the motivational system which drives distress calling.

4.2. Crowing with Sexual Motivation

The female birds from Group TP began to crow in their home-cages from Day 29. Probably, these birds crowed with sexual motivation like sexually mature male quails. No birds from Group DHT, on the other hand, crowed in their home-cages by the end of the experiments in spite of the fact that they crowed in the recording chamber much more frequently than the TP-treated birds (see Figures 1(b) and 2(b)). The level of circulating androgens in the DHT-treated birds were not insufficient because the growth of their cloacal glands was significantly more than that of the TP-treated birds (growth of their cloacal glands was significantly more significant as compared to the TP-treated birds). The female birds from Group TP began to crow in their home-cages when compared with the TP-treated birds in the present study crowed much less frequently in their home-cages.

It is, however, noticeable that the TP-treated female birds in the present study crowed much less frequently in their home-cages when compared with the TP-treated male birds in our above-mentioned previous study [9]. In the TP-treated males, for example, the frequency (mean ± s.e.) of home-cage crowing (number/30 min) at Day 31, 34, 38 and 42 was 27.43 ± 7.30, 45.00 ± 8.98, 58.67 ± 8.77 and 74.25 ± 16.74, respectively. Three of the 8 TP-treated male birds had already crowed in their home-cages by Day 25, much earlier compared to the intact or vehicle-treated control birds. Taken together, these data suggest that although crow vocalization itself can be induced solely by AR activation, ER activation is indispensable to motivate crowing as a courtship call.

Figure 4

In conclusion, the present study combined with our previous study in the male quails suggests that crowing, if it is restricted to sexually motivated one, is sexually differentiated to a substantial degree although not an “all-or-none” phenomenon.
REFERENCES


