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The induction of aromatase and sexual behaviour by testosterone in male and female Japanese quail: a dose-response study

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The aromatization of testosterone into estradiol is a critical step in the activation of sexual behaviour by testosterone. In the Japanese quail, it has been shown that activating effects of testosterone on sexual behaviour can be mimicked by estradiol administration and blocked by concurrent treatment with the aromatase inhibitor, androstatrienedione (1). We demonstrated recently that the aromatase of the preoptic area is sexually differentiated: it is higher in males, which copulate in response to testosterone treatment than in females which do not show this behavioural response (2). This enzyme activity is decreased by castration and restored to levels typical of sexually mature birds by testosterone treatments (3). It is interesting that the inducibility of the preoptic aromatase activity by testosterone is significantly higher in males than in females, a fact which, due to the critical role of this enzyme in the activation of copulation, could in part explain the sexual dimorphism in behaviour (3). The present experiment was undertaken to provide quantitative parametric data on this sexually differentiated enzyme inducibility and relate it more closely to the behavioural activation.

Materials and methods: Male and female Japanese quail *(Coturnix coturnix japonica)* were gonadectomized at the age of 4 weeks or submitted to a sham operation (see reference 4 for details of surgical techniques and housing conditions). At the age of 6 weeks, gonadectomized males and females were implanted with Silastic capsules filled with testosterone or left empty as control. Fourteen experimental groups were defined in this way from the type of surgery they had undergone and length of Silastic capsules they received, namely gonadectomized birds receiving testosterone implants of 2 mm ($n = 4$ males and 3 females), 5 mm ($n = 5$ males and 3 females), 10 mm ($n = 5$ males and 4 females), 20 mm ($n = 5$ males and 4 females) or 40 mm ($n = 4$ males and 4 females) or gonadectomized birds receiving empty (40 mm) implants as control (group 0; $n = 4$ males and 4 females) or sham-operated intact birds (group I; $n = 7$ males and 8 females). Two weeks later the sexual behaviour of these birds was quantified during three successive tests of 5 min in each taking place on successive days. In each test, a bird was presented to a sexually mature female and the frequency and latency of the following patterns of sexual behaviour were recorded: neck grab, mount attempt, mount and cloacal contact movement (see reference 5 for description). If a behaviour was not observed during one test, it was given a latency of 300 s for calculation purposes. Behavioural scores were summed over the three tests. The complement of the latency scores (900− total latency during the three 5 min tests) were calculated to provide scores directly proportional to behavioural activation. The frequency of crowing (an androgen-dependent vocalization) was also recorded for each bird during six sessions of 5 min each, taking place during the second week after implantation. At the end of that week, the cloacal gland (androgen-dependent structure) of each bird was measured and all birds were sacrificed. Their brain was immediately dissected and frozen on dry ice. The preoptic area and anterior hypothalamus (corresponding to areas P1 and P2 in reference 4) were then dissected (mean weight ($\pm$ SD) = 10.95 $\pm$ 1.35 mg) and homogenized in 500 μl buffer. The aromatase activity (transformation of testosterone into estradiol) was then measured in 100 μl fractions of these homogenates by an *in vitro* radioenzymoassay previously described and validated (6) in the presence of [^{3}H]-testosterone at a concentration of 22.5 nM. Protein content of the homogenates was measured by the coumassie blue method (7) and was equal to 1.39 $\pm$ 0.25 mg/ml (i.e., the ratio of protein to fresh weight was 6.35%; the correlation between fresh weight and protein content of the samples was $r = 0.77$, $p < 0.001$). All data were analyzed by two way analyses of variance (ANOVA) with sex and treatments as factors.

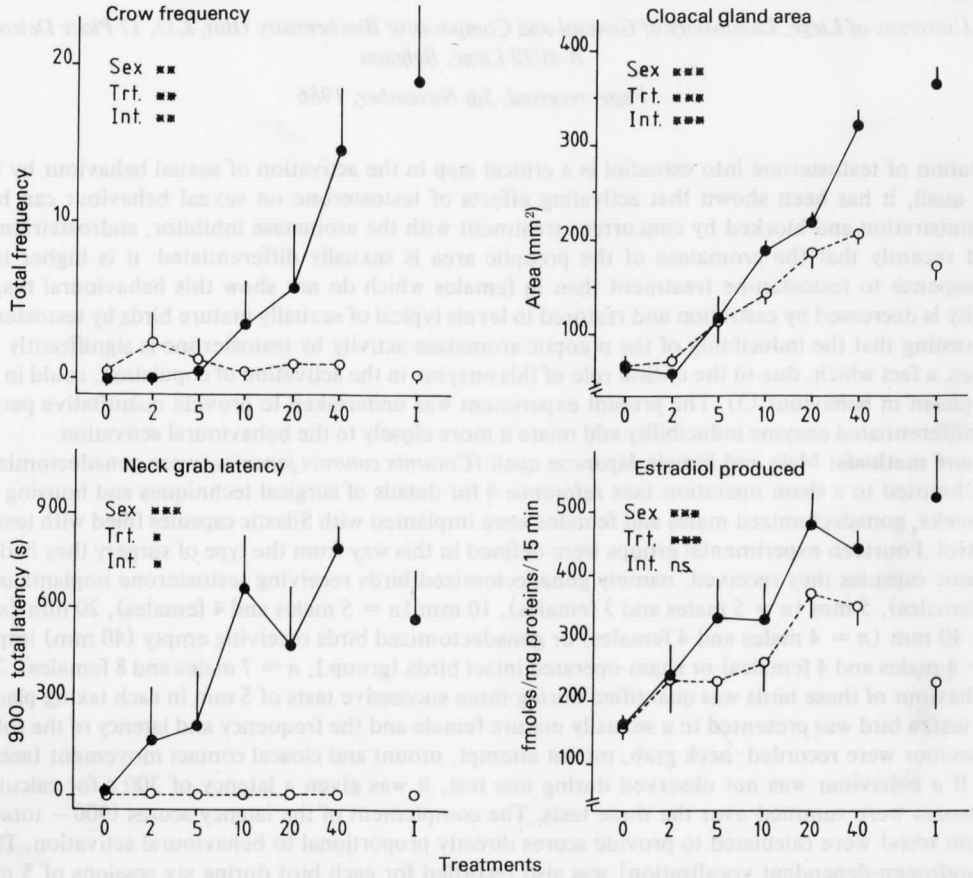
The total latencies and frequencies of the four sexual behaviour patterns were significantly correlated with each other (r = at least 0.84 in each case, $p < 0.001$). Data for neck grab only will thus be presented to avoid redundancy.

Results: In gonadectomized males, testosterone induced sexual behaviour, crowing and cloacal gland growth in a dose-dependent manner. In birds treated with the largest dose of testosterone, these androgen-dependent variables reached levels typical of mature intact males. Crowing and cloacal gland growth were activated to a lesser extent by testosterone in females. The hormonal treatments induced no male-type sexual behaviour in females. These findings were statistically confirmed by the two-way ANOVA's which revealed significant effects of sex and treatment on these variables as well as significant interactions between the two factors.

The two-way ANOVA also revealed a significant effect of sex and treatment of the birds on the aromatase activity: it was higher in males than in females irrespective of the surgical or hormonal treatment (with the exception of gonadectomized birds). The aromatase activity was also decreased by gonadectomy and increased by testosterone treatment. In gonadectomized testosterone-treated birds, the difference was present at each dose level above 2 mm so that the two-way ANOVA revealed a significant sex effect in all testosterone-treated birds (analysis on groups from 5 mm to 40 mm: $F = 7.57$, $p = 0.01$).

Discussion: This experiment confirms and extends previous results showing that testosterone differentially affects sexual behaviour, crowing and cloacal gland growth in gonadectomized male and female quail. This sexual dimorphism is especially pronounced in the case of sexual behaviour: absolutely no response was observed in females following treatment which restored behaviour of males to the level seen in sexually mature birds. In contrast, the sexual dimorphism affecting aromatase inducibility by testosterone was not an all or none phenomenon: inducibility was higher in males than females for every dose of testosterone except 2 mm but enzyme activity was nevertheless increased in testosterone-treated females. A previous experiment had shown that the inducibility of aromatase activity was higher in males than females following treatment with a high dose of testosterone (40 mm Silastic implants; see reference 3). The present study shows that it is not only the maximal enzyme activity which is sexually differentiated but rather the response of the enzyme to the testosterone treatment throughout the range of doses which were used. This suggests that it is not only the number of

aromatase positive neurons or maximal response of individual neurons which is differentiated but rather the whole mechanism of enzyme inducibility in response to testosterone. This finding could be a lead into the analysis of the molecular mechanisms underlying this sexual dimorphism.



*Effects of castration and treatment with testosterone on crowing, cloacal gland growth, sexual behaviour and preoptic aromatase activity in the male (●——●) and female (○– – –○) quail (means ± SEM). The data were analyzed by two-way analyses of variance and the significance of the main effects and their interaction is reported in the Figure. Sex = effect of sex; Trt = effect of treatments; Int = sex × treatment interaction. *p < 0.05; **p < 0.01; ***p < 0.001, ns = p > 0.05.*

This study also clarifies the relationships between induction of aromatase activity and sexual behaviour. The percentage of birds which showed at least one neck grab, mount attempt or cloacal contact movement during at least one of the tests increased regularly with the dose of testosterone and a good behavioural activation appeared only for doses between 5 mm and 10 mm of testosterone (2 mm: 50, 25 and 0% of birds showing neck grab, mount attempt, cloacal contact movement, respectively; 5 mm: 60, 60, and 40%; 10 mm: 100, 100 and 100%). This dose corresponds to an induction of aromatase activity of approximately 100 % as compared to gonadectomized birds (at least 300 fmol/mg proteins/15 min). There is a good parallelism between the induction of aromatase activity and the activation of sexual behaviour and it seems that a doubling of the enzymatic activity could be a minimal increase required for behavioural induction. This, however, only represents a necessary but not sufficient condition permitting the activation of the behaviour as revealed by the fact that females treated with 20–40 mm of testosterone did not show sexual behaviour despite the fact that their aromatase activity was increased above the critical level. This idea is also supported by the fact that in the groups given 2 mm and 5 mm of testosterone which showed no male behaviour, the birds had in some cases levels of aromatase higher than 300 fmol/mg proteins.

In conclusion, this study demonstrates that testosterone increases aromatase activity in the preoptic area in a dose dependent manner. This inducibility is sexually differentiated throughout the range of doses which were studied and it is correlated with the differential activation of sexual behaviour. Together with previous experiments, these data suggst that the preoptic aromatase activity is a limiting factor in behavioural activation but that other factors still to be determined (such as androgens directly acting on the brain) are implicated in the control of behaviour.

1. Adkins, E.K., Boop, J.J., Koutnik, D.L. *et al.* (1980) *Physiol. Behav.*, **24**, 441-446
2. Schumacher, M. and Balthazart, J. (1984) *Prog. Brain Res.*, **61**, 51-59
3. Schumacher, M. and Balthazart, J. (1986) *Brain Res.*, **370**, 285-293
4. Schumacher, M. and Balthazart, J. (1984) *Horm. Behav.*, **18**, 298-313
5. Adkins, E.K. and Adler, N.T. (1972) *J. Comp. Physiol. Psychol.*, **81**, 27-36
6. Schumacher, M., Contenti, E. and Balthazart, J. (1984) *Brain Res.*, **305**, 51-59
7. Bradford, M.M. (1976) *Anal. Biochem.*, **72**, 248-254

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