

Sexual differentiation and hormonal control of the sexually dimorphic medial preoptic nucleus in the quail

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We recently identified a sexually dimorphic nucleus in the preoptic region of the Japanese quail, the medial preoptic nucleus (POM), which is significantly larger in males than in females. In the present study, we investigated the hormonal control of this morphological neuroanatomical difference and the possible relationships between the sexual dimorphism in POM volume and in copulatory behavior. Treatments which are known to affect sexual behavior were thus applied to different groups of birds and the POM volume was then measured. In one experiment, male and female quails were either gonadectomized, gonadectomized and treated with testosterone or left intact. The larger size of the POM in males was confirmed and treatments significantly affected the nucleus size which was decreased by gonadectomy and restored by testosterone treatment in both sexes to a level similar to that seen in intact males. In two other experiments, eggs were injected with estradiol benzoate on day 9 of incubation and the POM volume was measured in adulthood either in intact birds or in gonadectomized birds receiving a replacement therapy with testosterone. Despite the fact that estradiol benzoate treatment completely suppressed copulatory behavior, it did not affect the volume of the POM or slightly increased it. These data thus show that the POM volume is controlled by testosterone levels in adulthood and could thus be an interesting model for the study of the effects of steroids on the brain.

INTRODUCTION

In the quail, like in many avian and mammalian species, many aspects of reproduction are sexually differentiated. Namely, it has been shown that the male-type copulatory behavior is activated by testosterone in males but not in females^{2,7}, plasma gonadotrophin levels are higher in males than females^{24,31}, noradrenaline concentration in the preoptic area (POA) is higher in females than in males¹⁹ and aromatase activity is higher in male POA than in female^{24,27}.

In mammals, sexually dimorphic structures have been identified in the POA of many species including rat¹², ferret³⁰, gerbil⁸, guinea pig¹³ and man²⁹. In birds, major sexual morphological differences have

been identified in the song controlling nuclei¹⁸, but, so far, no dimorphic structures have been identified in the hypothalamus^{20,21}. We recently examined the POA for morphological correlates of the behavioral and physiological differences which had been found in quail. We identified a sexual dimorphism in the volume of the medial preoptic nucleus (POM). This periventricular mass is significantly larger in males than in females³³. In some mammals the gross volume of the sexually dimorphic structures in the POA is affected by hormonal manipulation in adulthood (gerbil: ref. 8) while in other species the hormonal environment during neonatal life seems to play a major role in the control of these sexually dimorphic structures¹². In this study we have examined the possible relationships between the sexual dimorphism in

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POM volume and in copulatory behavior in the quail. Treatments which are known to affect sexual behavior (adult castration and testosterone replacement⁷; injection of estradiol benzoate in the egg¹) were applied to different groups of birds and their effects on behavior were verified. The brain of each animal was then collected and examined for the POM volume.

MATERIALS AND METHODS

Subjects

All experiments presented in this paper were carried out on the Japanese quail (*Coturnix coturnix japonica*). Subjects were bought at the age of 3–4 weeks from a local breeder. From their arrival in the laboratory till the end of the experiment, they were exposed to a photoperiod of 16 h light and 8 h dark with food and water available ad libitum. Until the beginning of the experiments, birds were kept in heterosexual groups; during the experiments they were isolated in individual cages. Birds were gonadectomized or sham-operated under total anesthesia (Hypnodil, Janssens Pharmaceutica, Belgium; 15 mg/kg b. wt.) through a unilateral incision behind the ribs on the left side. Both testes were taken in males through this incision; in females, the left ovary only was removed as the right ovary is not developed and does not regenerate even after castration on the left side¹¹. Eggs were obtained from a local breeder and incubated in the laboratory.

Hormone treatments

Estradiol benzoate (EB) was injected into the egg after being dissolved in 50 μ l sesame oil. Control eggs were injected with an equal volume of oil. Injections were carried out with a sterile syringe at the small end of the egg. The hole in the shell was then sealed with melted paraffin. Adult birds which had to be treated with testosterone (T), received 60 mm (3 $\times$ 20 mm) of silastic implants (Dow Corning tubing, number 602-252; i.d. 1.57 mm, o.d. 2.41 mm) filled with crystalline T (Sigma T-1500). Control birds received empty implants of the same length. These capsules were implanted s.c. in the neck region.

Behavioral tests

On several occasions, birds were tested for sexual behavior in a test arena (60 $\times$ 40 $\times$ 50 cm) the floor of

which was covered with sand. A stimulus egg-laying female was introduced in the arena and 30 s later the experimental subject was added. Its behavior was then observed for 5 min and the frequency of the following behavior patterns was recorded: neck grab, mount attempt, mount, cloacal contact movement and strut (see ref. 2 or 14). After the test, the area of the cloacal gland (an androgen-dependent structure) was measured with a calliper to the nearest millimeter (area = largest length $\times$ largest width). Body weight was recorded to the nearest gram.

Histological procedure

At the end of the experiments, birds were heparinized by injecting 100 μ l of heparin solution (30 mg/ml) into the wing vein and then were deeply anesthetized (irreversibly) with an overdose of Hypnodil. They were perfused with saline solution followed by either paraformaldehyde–glutaraldehyde (Expt. 1; PAF 4%–GA 0.5%) or Bouin's fixative (Expt. 2 and 3). Heads were postfixed for about 48 h in the same fixative and then brains were dissected, dehydrated and embedded in Histosec (Merck). More shrinkage was observed in Bouin's than in aldehyde-fixed material so that no comparison of volumes could be made across the experiments. Specimens were sectioned at 30 or 50 μ m according to the experiment and sections were Nissl-stained with Toluidine blue. Slides were then coded so that subsequent data analysis could be done with the experimenter not knowing the sex or treatment of the brains under study. When appropriate, completeness of gonadectomy and presence of the silastic implants were controlled. Birds with gonadal remnants or without implants were removed from the analysis and the numbers reported here only reflect those birds which were retained.

Morphometrical analysis

The volume of selected nuclei (POM and nucleus rotundus thalami, nRT) was calculated by means of a BASIC program running on an Apple IIe computer provided with a graphics tablet. This program measures the area of the traced nucleus in each section by means of coordinates recorded by the graphic tablet. Drawings on the tablet were obtained either by camera lucida or directly on the video screen connected to a Leitz Laborlux microscope via a Sony TC camera. The volume was calculated according to the for-

mula n. 1 published by Uylings et al.³². This method provides a value of the volume of nuclei which is not affected by the thickness of the sections and even permits to calculate volumes when a few sections are missing provided that the changes in area between successive sections are linear. In any case, no statistical comparison was ever made between data collected from sections with different thickness.

Due to the difficulty in recognizing the boundaries of preoptic nuclei in Nissl-stained sections, the volumes were calculated separately from the drawings made independently by 3 (two for Expt. 3) observers for each specimen. During Expt. 1, the correlation between these 3 sets of measures was calculated and ranged between 0.62 and 0.81 ($P < 0.001$). All conclusions presented in this paper were obtained by the analysis of the 3 sets of values as well as of the data collected by each separate observer with only one exception as mentioned in the text. The same procedure has been used previously in a similar study⁸. The volume of the POM was measured on both sides and the total volume is presented here. In some experiments and for some groups of birds, a left-right asymmetry in the volume of the POM was observed. However, the difference was always very small (2–4%); it was not reliably observed in the different experiments nor by the different observers. Additional experiments are now in progress to test for the influence of the observation technique upon the observed asymmetry. As a control for non-specific changes in brain volume, the volume of the nRT was measured on one side. This nucleus was chosen because it is close to the hypothalamus and its boundaries are clearly delimited by fiber tracts. There is thus no ambiguity in defining the cross-sectional area of the nucleus so that measurements were taken by only one observer (G.C.P.).

Statistical tests

Mean values of the volumes measured by the 3 observers were used for the statistical analysis. Analyses were also run on data from each individual observer and, unless otherwise mentioned, led to the same conclusions. Two-way analysis of variance was used to evaluate the effect of sex of the birds, hormonal treatments and their interaction. Differences were considered significant at $P < 0.05$. Behavioral data were analyzed by non-parametrical statistics (Krus-

kall-Wallis analysis of variance, Mann-Whitney *U*-test).

EXPERIMENT 1

This first experiment was carried out to test whether the sex difference in POM volume previously seen in intact birds was related to their different hormonal milieu or rather represented a stable sexual difference presumably organized by neonatal hormones. Birds of both sexes were gonadectomized and treated with T.

Procedure

The experiment was carried out on 38 Japanese quails bought at the age of 3 weeks. One week later all birds were either gonadectomized or sham-operated. At the age of 6 weeks they were weighed, their cloacal gland was measured and they were randomly separated into 6 experimental groups defined as follows:

Control males ($n = 7$): sham-gonadectomized males (S).

Castrated males ($n = 7$): gonadectomized males with no hormone treatment (CX).

Testosterone-treated males ($n = 4$): castrated males treated with 60 mm silastic implants of T (CX+T).

Control females ($n = 9$): sham-gonadectomized females (S).

Ovariectomized females ($n = 5$): gonadectomized females with no hormone treatment (OVX).

Testosterone-treated females ($n = 6$): ovariectomized females treated with 60 mm silastic implants of T (OVX+T).

Eighteen and 19 days after implantation, birds were tested twice for sexual behavior. On day 19, cloacal gland and body weight were also measured. On day 20, one blood sample (2 ml) was collected from the wing vein of each bird using heparinized syringes. Samples were centrifuged and the plasma was stored at -20°C until assayed for T and luteinizing hormone by the methods described and validated by Delville et al.⁹ and Follett et al.¹⁰. Birds were sacrificed on day 21–22 and their brain processed for histology.

TABLE 1

Plasma hormones levels, cloacal gland area and sexual behavior (total frequencies during the 2 behavioral tests) in the 6 groups of birds from Expt. 1

Data shown for plasma hormones and cloacal gland area are means $\pm$ S.D. They were analyzed by two-way ANOVA which separately assessed the effects of sex, hormonal treatments (trt) and their interaction (int). These results (F ratio and probabilities) are shown on the right part of the table. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Behavioral data were analyzed by Kruskal–Wallis ANOVA followed by Mann–Whitney U -test. Comparisons with the male S group (**2 $P < 0.01$) or with the corresponding group of the other sex (*2 $P < 0.01$) are shown beside the median of each group. MA, mount attempts; CCM, cloacal contact movements.

Variable	Males			Females			ANOVA		
	S	CX	CX+T	S	OVX	OVX+T	sex	trt	int
Plasma hormones (ng/ml)									
T	1.51 $\pm$ 0.69	0.29 $\pm$ 0.18	1.28 $\pm$ 0.41	0.38 $\pm$ 0.20	0.19 $\pm$ 0.07	1.21 $\pm$ 0.46	9.92**	18.64***	6.36**
LH	2.56 $\pm$ 1.23	14.43 $\pm$ 5.04	2.26 $\pm$ 2.62	0.60 $\pm$ 0.48	7.53 $\pm$ 4.47	4.84 $\pm$ 3.96	4.14*	30.90***	7.06**
Cloacal gland area (mm ²)									
day 0	212 $\pm$ 53	45 $\pm$ 5	44 $\pm$ 9	87 $\pm$ 65	47 $\pm$ 6	43 $\pm$ 11	11.42**	31.75***	11.31***
day 19	356 $\pm$ 95	52 $\pm$ 10	328 $\pm$ 43	134 $\pm$ 38	56 $\pm$ 24	254 $\pm$ 39	31.53***	70.82***	14.82***
Sex behavior (median frequency and range)									
MA	14 (0–24)	0 (0–0)**	13 (9–23)	0 (0–0)***	0 (0–0)**	0 (0–0)***	$H = 31.65***$		
CCM	4 (0–7)	0 (0–0)**	1.5 (0–8)	0 (0–0)***	0 (0–0)**	0 (0–0)**	$H = 27.83***$		

Results

In agreement with previously published papers, T treatment activated sexual behavior in males but not in females (see Table I) despite the fact that the hormone implants produced similar levels of T in both sexes.

In T-treated birds, the T level was comparable to that seen in intact mature males. Cloacal gland growth was also stimulated more in males than in females by the steroid treatment. All the variables under study were significantly affected by the sex and treatment of the bird (see Table I for details). At the end of the experiment the sham-operated birds were fully mature: males had developed testes and all females were laying eggs. Body weight was not significantly affected by treatment, nor by sex, nor by their interaction, except that females were heavier than males at the start of the experiment. The volume of the POM was also significantly affected by the sex and hormonal treatments (see Fig. 1).

The overall ANOVA confirmed that the nucleus was larger in males than females ($F = 7.27$, $P < 0.01$) and that it was affected by the hormonal treatments ($F = 7.77$, $P < 0.002$). No significant interaction between the two factors (sex and treatment) was observed. The same conclusions were reached in the separate analysis of the data collected by the 3 observers, with the exception that one observer (C.V.-

P) did not detect a significant sex effect. Partial analyses were also carried out by two-way ANOVA to compare hormonal treatments two by two (sham vs gonadectomized, gonadectomized vs gonadectomized plus T). Both analyses confirmed the sex difference ($P < 0.05$ in both cases), the effect of T on gonadectomized birds was shown to be extremely significant ($P < 0.001$), but the effect of gonadectomy did not reach significance ($F = 2.26$, $P = 0.142$).

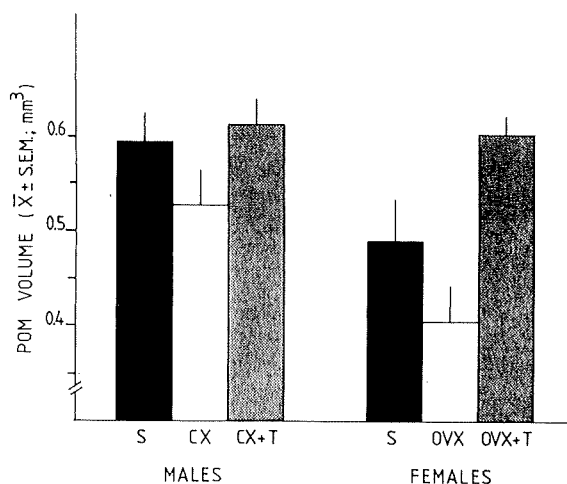


Fig. 1. Volume of the POM in male and female Japanese quail which were either left intact and sham-operated (S), castrated (CX) or ovariectomized (OVX), or gonadectomized and treated with testosterone (CX+T and OVX+T).

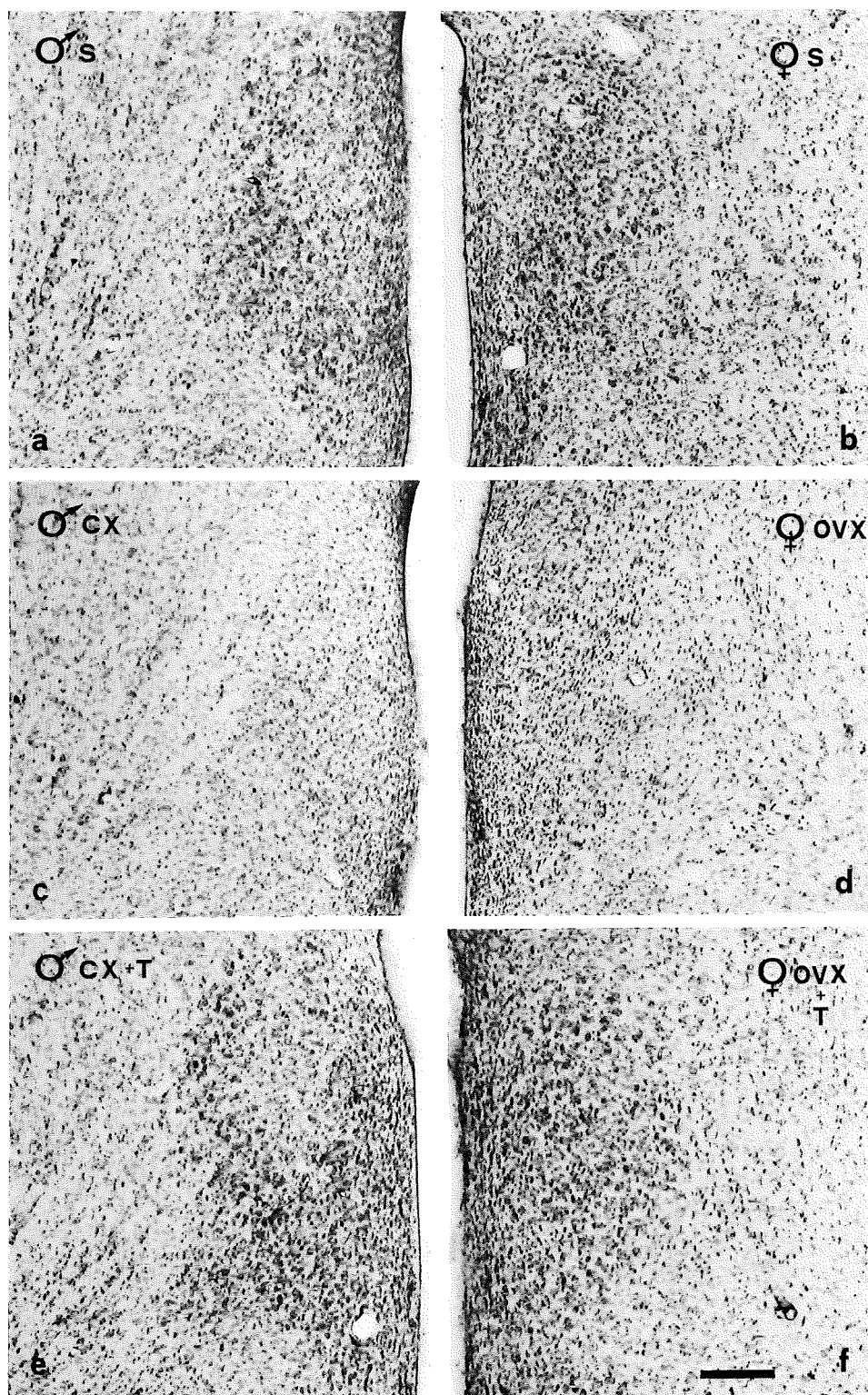


Fig. 2. Hormonal effects on the morphology of the Japanese quail POM. Representative coronal sections through the rostral POM in birds from Expt. 1. (a) Sham-operated male; (b) sham-operated female; (c) castrated male; (d) ovariectomized female; (e) castrated male treated with T; (f) ovariectomized female treated with T. The Nissl staining of the POM shows a marked decrease both in castrated males and ovariectomized females (c, d). The staining intensity increases greatly after treatment with T (e, f). Bar = 200 μ m.

These changes in total volume of the POM were paralleled by changes in staining (see Fig. 2).

The Nissl staining was much lighter in gonadectomized animals making the nucleus almost invisible in OVX females, but was restored by T treatment in both sexes to levels normally seen in intact males. For control, the volume of the thalamic nRT was analyzed by one observer. No significant effect of sex, nor treatment, nor their interaction could be detected.

EXPERIMENT 2

Expt. 1 showed that the POM volume could be affected by adult hormonal manipulations. Specifically it demonstrated that treatment of females with T produced POM volumes similar to those seen in intact males which suggested that this characteristic was not a stable sexual dimorphism organized by the neonatal hormone environment. EB treatment of the embryonic quail is known to have irreversible organizational effects on the brain¹; this treatment suppresses the sensitivity to the activating effect of testosterone on male copulatory behavior. We thus tested whether EB treatment of quail embryos had any permanent effect on the volume of the POM. In this way, we also tested for possible correlations between POM volume and presence of male copulatory behavior.

Procedure

Eggs ($n = 150$) were bought from the local breeder and incubated in the laboratory. On day 9 of incubation they were injected either with 25 μg of EB dissolved in 50 μl of sesame oil ($n = 75$) or with oil as control ($n = 75$). After hatching ($n = 18$ oil control, and 19 EB-injected) body weight was recorded at 0, 1, 2 and 6 weeks. Birds were isolated at the age of 6 weeks (5 EB and 11 control males, 4 EB and 6 control females) and their sexual behavior was recorded during one 5-min observation at 7 weeks of age. Their cloacal gland area was also measured on that day. Sacrifice and brain dissection took place two weeks later. The brains of 5 control and 5 EB males and of 6 control and 4 EB females were subjected to quantitative analysis of the volume of the POM and nRT.

Results

The treatment of embryos with EB produced the

expected effects on the sexual behavior and the cloacal gland growth. All control males showed mount attempt and cloacal contact movements (with one exception) during the behavior test. These behaviors were completely absent in the EB males and in both groups of females (see Fig. 3). EB treatment differentially affected cloacal gland growth in males and females: it was significantly decreased in males and not affected (slightly increased) in females this resulting in a significant interaction between the effects of sex and treatment ($F = 7.59$, $P < 0.011$; effects of sex and treatment are not significant). In control birds this structure was larger in males than in females (see Fig. 3).

Experimental treatment had no effect on body weight. Testes weight was slightly decreased in EB

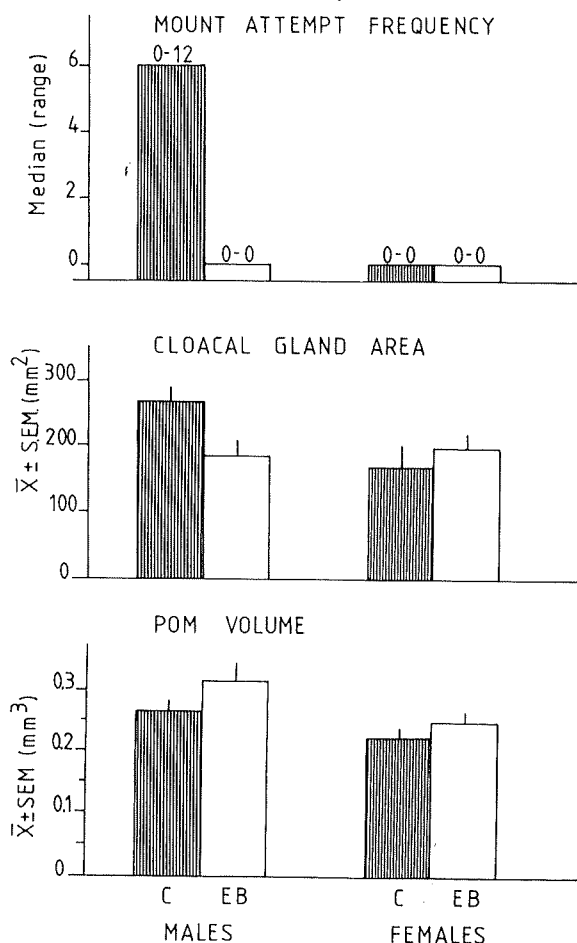


Fig. 3. Sexual behavior (frequency of mount attempts during one behavioral test), cloacal gland area and volume of the medial preoptic nucleus (POM) in male and female Japanese quail injected in egg with EB or oil as control (C) during Expt. 2.

males (5.52 ± 1.07 vs 6.27 ± 0.76 g) but this effect did not reach significance ($t = 1.36$, $P > 0.05$). At sacrifice all the control females, except one, had laid eggs (mean number 6.6) while none of the EB females had done so (Fisher's test: $2 P < 0.047$). In both groups, however, the ovary was mature and had big follicles larger than 10 mm in diameter (except for the one control female which did not lay eggs).

The ANOVA revealed a significant effect of sex and treatment on the volume of the POM. The nucleus was larger in males ($F = 14.41$, $P < 0.001$) and increased by EB treatment in both sexes ($F = 6.07$, $P < 0.024$; no significant interaction observed). In control birds, the nRT volume was slightly larger in females than in males but the difference was not significant.

EXPERIMENT 3

The injection of EB in eggs showed that the POM volume could be affected also by prenatal hormonal manipulations, but the results of this experiment could not receive a final interpretation due to the fact that the presence of gonads in posthatching birds could affect the POM volume. The treatment of embryos with EB could have affected the testosterone secretion by the gonads in a permanent way, so that the increased POM volume would have reflected the higher plasma testosterone level (activational effects of hormones) rather than the permanent effect of EB in the brain (organizational effects of hormones). For this reason, we started the present experiment in which EB-injected embryos were gonadectomized and treated with T before the behavioral tests and anatomical investigations in order to standardize the adult hormonal conditions.

Procedure

Eggs were bought and treated in the same way as in Expt. 2 (50% injected with $25 \mu\text{g}$ of EB, 50% injected with sesame oil on day 9 of incubation). Four weeks after hatching all birds were gonadectomized. One week later they were put in isolation cages and implanted with 60 mm of silastic capsules of T. At that age, a total of 67 birds were available and were separated into 4 experimental groups defined as follows: control males ($n = 20$), EB males ($n = 20$), control females ($n = 15$), EB females ($n = 12$). All birds

were either oil- or EB-injected in egg and gonadectomized and T-implanted. At the age of 7 weeks, all these birds were tested 3 times for sexual behavior on 3 consecutive days. After the last test their cloacal gland was measured. Sacrifice and brain dissection took place 3 days later. Body weight was measured at the age of 4 and 7 weeks.

Results

This experiment confirmed the demasculinizing effect of EB injection in eggs on male copulatory behavior. During the 3 tests, 19 out of 20 control males showed mount attempts while only 7 out of 20 did it in the EB group (Fisher's test, $2 P < 0.0001$). The same

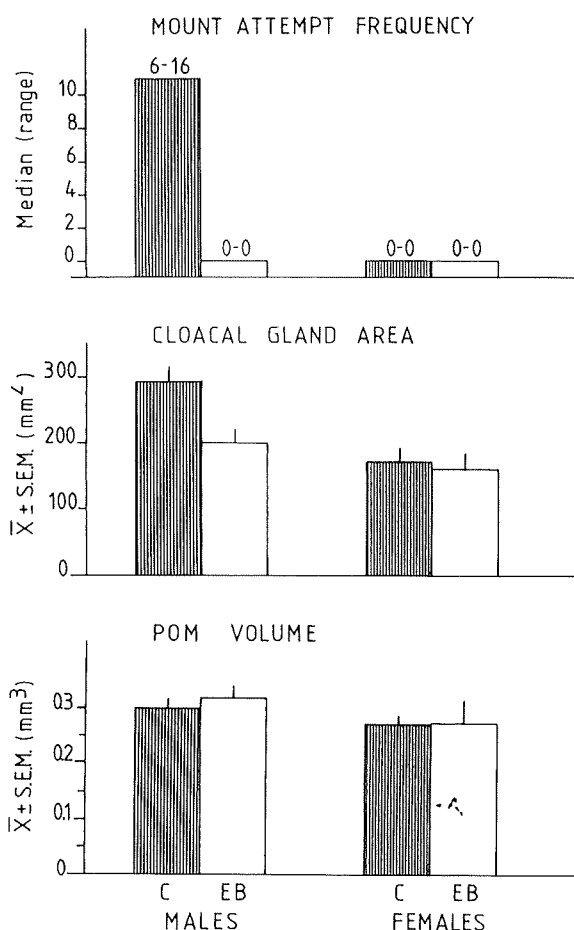


Fig. 4. Sexual behavior (frequency of mount attempts during 3 behavioral tests), cloacal gland area and volume of the medial preoptic nucleus (POM) in male and female Japanese quail injected in egg with EB or oil as control (C) during Expt. 3. All birds were gonadectomized at 4 weeks after hatching and all were implanted with T capsules.

results were obtained for cloacal contact movements (16/20 active in control males, 2/20 active in EB males; $2 P < 0.00002$). No copulatory behavior was seen in females. From these data subpopulations of animals were selected for histological analysis (7 control and 6 EB males, 7 control and 5 EB females; the EB males selected were all sexually inactive and the control males were all sexually active). Frequency of mount attempts, cloacal gland area and POM volume for these birds is shown in Fig. 4.

Sexual behavior was only seen in control males. Cloacal gland area was significantly affected by sex ($F = 42.38$, $P < 0.001$), by treatment ($F = 16.63$, $P < 0.001$) and by their interaction ($F = 9.22$, $P < 0.005$). This area was larger in males than females and was decreased by EB treatment in eggs. Body weight was not affected by treatment, nor by sex. POM volume was not significantly affected, although a marginal increase was observed following EB treatment especially in males ($F = 3.77$, $P = 0.062$). The volume of the nRT was also similar in the 4 groups of birds.

DISCUSSION

We have previously shown that the medial preoptic nucleus (POM) of the quail is a sexually dimorphic structure^{22,33}. This nucleus is located in an area which is known to be involved in the control of male copulatory behavior in birds⁶ and in mammals¹⁷, and recent autoradiographic studies show that it accumulates estradiol (J.T. Watson and E. Adkins-Regan, personal communication, June 1986). Sexually dimorphic nuclei have also been identified in several species of mammals and the hormonal control of these neuroanatomical differences have been studied in a few cases (see Introduction).

In the present study we have investigated the hormonal dependence of the sexual dimorphism in quail POM volume by manipulating adult and embryonic hormonal conditions. The hormonal treatments which were performed are known to influence profoundly copulatory behavior of the quail²⁶. In this way, we were testing the idea that regulation of POM volume is parallel to regulation of copulatory behavior. The collected data confirm results of previous experiments on sexual behavior. In male quail castration suppresses copulatory behavior which is restored by T treatment (Expt. 1; ref. 7). Treatment of

male embryos with EB on day 9 of incubation totally abolishes their sexual behavior (Expt. 2 and 3; ref. 26). Mount attempts and cloacal contact movements are completely absent in females irrespective of their hormonal condition (Expt. 1–3; refs. 2, 7). Cloacal gland area shows similar hormonal control: it is decreased by EB in egg¹ and stimulated by T treatment in adulthood⁷. Females always have smaller glands than males even when treated with T.

These adult and embryonic treatments with hormones also affected the volume of the POM in a specific way, as demonstrated by the fact that the volume of the nRT, a closely located thalamic area, was not changed. Hormonal regulation of POM volume and copulatory behavior was not always similar. Castration decreased and T treatment increased the size of this preoptic nucleus in male in parallel with changes in sexual behavior frequency. However, the effect on morphology was seen in both sexes (T increased female POM to levels seen in intact males) while T stimulated copulation in males but not in females (Expt. 1, confirmed in the control groups of Expt. 3). In addition EB treatment during incubation did not affect (Expt. 3) or slightly increased (Expt. 2) POM volume in male, while it completely suppressed male copulatory behavior. This EB-induced increase was observed only in Expt. 2 in which birds were not gonadectomized. It is thus possible that EB affected the hormonal condition of the adult birds so that the neuroanatomical difference reflected activational rather than organizational effects of steroids. It is nevertheless unlikely that plasma T levels were increased in the EB-treated males by comparison with their controls because testes weight did not show such an increase (small decrease was rather observed) and the cloacal gland area was much smaller in EB than in control subjects (but this could reflect changes in sensitivity to T rather than changes in plasma levels of T; ref. 25).

The increase in POM volume observed after EB injection during Expt. 2 can receive no physiological explanation. It is known that female galliforms have higher plasma levels of estradiol than males during incubation (in chicken³⁴; in quail, *in vitro* data²³) and still the volume of the POM is smaller in females than in males. We thus suspect that the levels of estradiol after the experimental procedure were supraphysiological and induced variations in neuroanatomical

structures which normally are not observed under the influence of endogenous steroids. This interpretation is also supported by the observation that egg laying was completely blocked in EB-injected females. This effect has already been observed by Adkins-Regan³ and seems to be related to oviduct abnormalities following overexposure to estrogens.

To our knowledge the effects of both neonatal and adult hormonal manipulations on the volume of sexually dimorphic structures in the POA have been studied only in the rat and in the gerbil. In the rat, the development of the sexually dimorphic nucleus (SDN-POA) is (perhaps totally) determined by the hormonal environment perinatally^{5,12,15}. The volume of the SDN-POA in the rat does not seem to be sensitive to the adult steroid levels¹², although its neurons clearly accumulate steroids as shown by autoradiographic studies¹⁶. In guinea pigs it also seems that perinatal hormones play a major role in the control of the dimorphic part of the medial POA because the sex difference in volume is seen in intact as well as gonadectomized adults¹³. However, manipulations of the neonatal hormonal environment have not been performed to confirm that conclusion. By contrast, in the gerbil we know that adult T levels influence the morphology of the sexually dimorphic area⁸ and neonatal treatment with T has similar effects³⁵. It thus appears that in mammals the sexual difference in the volume of the dimorphic structures in the POA is largely controlled by the neonatal environment with a contribution of the adult hormones which could be variable from one species to another. In quail the present experiments lead to a rather opposite conclusion: the volume of the POM is strongly affected by adult T levels, while treatment with EB in the egg has no or marginal effects. Whether this represents a true species difference or a difference in the structure considered and/or the specific experimental procedures still needs to be evaluated. It must be mentioned that effects of EB only were tested in the quail embryos and no data are available on the possible effects of embryonic treatments with T on the POM volume in adulthood. This experimental approach was chosen because the organizing effects of steroids on brain and behavior in quail which have been previously identified are known to be estrogen-dependent: even when T is active it is through aromatization⁴.

The present data do not permit to completely rule out the possibility that embryonic or neonatal hormones (namely T) have organizational effects on the POM. We showed that there is no sex difference in POM volume in gonadectomized birds treated with T and that EB injection on day 9 of incubation has little effect on this parameter, but there was some suggestion in the data that the POM volume could be larger in castrated males than in ovariectomized females. This difference was not significant but this could be related to the limited sample size and should be further investigated. Other neonatal manipulations of hormones, which also affect sexual differentiation of behavior, should also be considered such as for example neonatal gonadectomy²⁵.

We thus have identified a nucleus in the POA which is sexually differentiated and hormone-sensitive in its gross volume. Other aspects of the POM are also probably dimorphic and hormone-dependent such as cell size and staining properties and these characteristics are now under study. The physiological role of this nucleus is at present unknown. The differential Nissl staining probably reflects differences in metabolic activity in the cell groups involved. In this context, it is interesting to remember that we showed recently that aromatization of T in the POA is also sexually differentiated in quail^{24,27}. The aromatase activity is also influenced by castration and T treatment²⁷ and recent data suggest that within the POA most of the enzyme activity is located within the POM²⁸. The metabolic changes in POM which are suggested by variations in Nissl staining could thus be related to the aromatization of T. As this process is critical for the activation of sexual behavior, the anatomical differences in POM could represent a morphological marker for biochemical processes involved in behavior control.

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