

A Two-Step Model for Sexual Differentiation^a

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During the last decade, the study of sexual differentiation in quail has been rewarding for two groups of reasons. First, the study of differentiation in an animal model quite different from rodents has for the first time progressed far enough to produce precise models and concepts. Results obtained in rodents can consequently be evaluated in a broader perspective and this should lead to redefinition of a number of notions. Quail research on differentiation also emphasizes that the rat is not a universal model for vertebrates and cautions against generalizations extrapolating rat data to primates or even humans (see Reference 1 for a detailed treatment).

Second, the quail has specific advantages which facilitate the study of sexual differentiation.² Embryonic development takes place in an isolated environment (the egg) which guards against external hormonal influences (from siblings *in utero*³; from the mother⁴) and at the same time facilitates treatment with exogenous hormones. Being precocial, young quail can be raised in isolation or at least in the absence of parental influence; parents could treat male and female (or hormone-treated and control) young differently and hence can be a source of confounding factors in an attempt to separate hormonal versus experiential effects (for a treatment of this problem in the rat, see the paper by C. L. Moore in this volume and Reference 5). Birds also show an extensive behavioral dimorphism, usually more pronounced than that in mammals. Finally, the lack of external genitalia in quail helps to avoid confusion between organizing effects of hormones on the brain and on peripheral tissues.

SEXUAL DIMORPHISM IN QUAIL

Sexual behavior is strongly differentiated in quail. The copulatory sequence (grab the neck feathers, mount, and cloacal contact movement) is seen only in males and never occurs in intact females.^{6,7} This behavior pattern is thus homotypical-male in the sense of Cheng and Lehrman.⁸ Squatting, which reflects sexual receptivity, is seen

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mainly in females but is also displayed occasionally by submissive males⁶ (Y. Delville and J. Balthazart, unpublished observations). Other behavior patterns are seen in both males and females (they are isomorphic; e.g., aggressive pecks) although with unequal frequencies (quantitative but not qualitative differences). Similarly, a number of morphological features are present in both sexes but show a quantitative dimorphism: males have a larger cloacal gland and larger sternotracheal (syringeal) muscles than females.⁷

These sex-related differences are not merely due to the different hormonal milieu to which each sex is exposed (i.e., ovarian versus testicular secretions). They are still observed in birds with regressed gonads (following exposure to short days) which are treated with adequate exogenous hormones: estradiol benzoate (EB) induces sexual receptivity in both males and females but testosterone propionate (TP) activates copulation in males only.^{8,9,10} Residual secretions from the photoregressed ovary do not explain the differential responsiveness to TP; gonadectomized females still do not show male behavior in response to testosterone (T) treatment.^{7,11} T also stimulates a larger growth of the cloacal gland and of the syringeal muscles in males than in females after gonadectomy or photoperiodic castration.^{8,7,9,12}

In quail it is thus mainly the male homotypical behavior which is sexually differentiated. By contrast, in rats and rodents in general, the sexual dimorphism in the responsiveness to exogenous steroids essentially concerns the female behavior, that is, the induction of lordosis by sequential treatment with EB and progesterone (P). In rodents, females are bisexual and show masculine and feminine behavior in response to adequate hormone treatments (TP and EB+P, respectively) while it is extremely difficult to induce males to show lordosis. A quantitative sex difference is nevertheless also observed in the masculine behavior of rodents (see References 1 and 13 for reviews).

In this paper, we shall summarize the available data on sexual differentiation in quail by focusing on two separate aspects of this process: its hormonal specificity and its time course. New data will be presented which show that female demasculinization is not completed during a short period of embryonic life as was thought previously but rather takes place during the embryonic life and the first few weeks after hatching. Directions for future work will also be discussed.

THE PROCESS OF SEXUAL DIFFERENTIATION

Before 1975, the ontogeny of these sexual differences in birds was poorly understood. A few experiments had shown that treatment of embryos with exogenous hormones suppresses mating behavior in the adult birds¹⁴⁻²⁰ (see Reference 21 for review). With one exception,²⁰ these studies gave no evidence that the behavioral changes reflect neural differentiation rather than alterations in gonadal maturation.

In the last 10 years, Adkins and collaborators have carried out a very nice group of studies which now permit us to construct a coherent picture of the process of sexual differentiation in quail.^{2,10,21,22}

They proved that differentiation of male behavior in quail is essentially a neural process. In all experiments, birds were tested for behavior after functional castration (exposure to short days²³) and activation of behavior by exogenous steroids. This showed that the absence of copulation in females was not related to inadequate activation but reflected the androgen insensitivity of the neural mechanisms involved in behavioral activation. By treating eggs with steroid hormones and various drugs,

it was shown that behavioral differentiation in quail mainly consists of the demasculinization of females by embryonic ovarian estrogens, i.e., the loss of the capacity to show male behavior even after adequate stimulation by testosterone. As a consequence, embryonic hormones were critical for the differentiation of females only and males could be considered as a "neutral" sex whose phenotype develops in the absence of hormonal control (the "anhormonal sex"). These propositions were derived from the observation that EB-treated male embryos do not show male-typical sex behavior even if injected with TP in adulthood.^{6,10} They are demasculinized by the exogenous hormone. It was then reasoned that this demasculinization was spontaneously taking place in untreated female embryos under the influence of ovarian estrogens. This was later supported by the demonstration that injection of an antiestrogen (C1-628) into eggs containing female embryos prevented their demasculinization.⁴ This experiment also supported the concept of the male as a neutral sex; antiestrogens had no effect on the behavior of males.

DEMASCULINIZATION AND HORMONAL SPECIFICITY

An unexpected finding in these studies on bird sexual differentiation was the apparent interchangeability of exogenous testosterone and estradiol as agents inducing demasculinization of males.⁶ This raised two critical questions:

- (1) What is the specificity of this response and what is the hormone normally active in intact females?
- (2) Why are males not demasculinized by their testicular secretions?

To answer the first question, Whitsett *et al.*²³ tested five different synthetic or natural estrogens (estradiol-17 β , estradiol-17 α , estrone, estriol, diethylstilbestrol) which were all capable of demasculinizing male quail at the dose of 2 μ g/egg. By contrast, T at the same dose had no significant effect on male behavior. A detailed dose-response study later confirmed that demasculinization of male quail requires much higher doses of androgens compared to estrogens: threshold doses for statistically significant effects were approximately 500 μ g for TP and 1 μ g for EB.¹⁰ These experiments provided a possible explanation as to why males were not demasculinized by their endogenous steroids: presumably circulating levels of testosterone were below threshold for demasculinization.

Studies on plasma levels of steroids in embryonic birds and on brain metabolism of steroids have confirmed this interpretation. Studies on the production of steroids by quail embryonic gonads cultured *in vitro* have demonstrated that testes do not produce more testosterone than ovaries while estrogen production (estradiol and estrone) is strongly dimorphic with much higher (5- to 10-fold) secretion in females.^{26,27} Although plasma levels of androgens and estrogens in both male and female embryonic quail are still unknown (T only has been measured in males²⁸), in the closely related chicken, sex differences in plasma testosterone (males > females²⁹; this sex difference was not found by Gasc and Thibier³⁰), estradiol (females > males³¹), and estrone (females > males³²) have been demonstrated which support the proposed model of differentiation (see also Reference 33 for plasma levels of steroids in embryonic chickens).

Furthermore, in an extensive series of experiments, Adkins-Regan *et al.*³⁴ showed that aromatization mediates the testosterone-induced demasculinization. This conclusion is supported by the observations that aromatizable androgens (T, TP, and androstenedione) demasculinized copulation while nonaromatizable androgens (dihydrotestosterone propionate and androstene) were inactive on this process, that the

antiestrogen, tamoxifen, blocks the TP-induced demasculinization, and that the aromatization inhibitor, androstatrienedione (ATD), blocks the demasculinization induced by TP but not that induced by EB. It has also been established that brains of male and female chickens aromatize androstenedione³⁵ (nothing is known about embryonic brains in quail specifically) and the embryonic chicken brain contains estrogen receptors (autoradiographic studies).³⁶

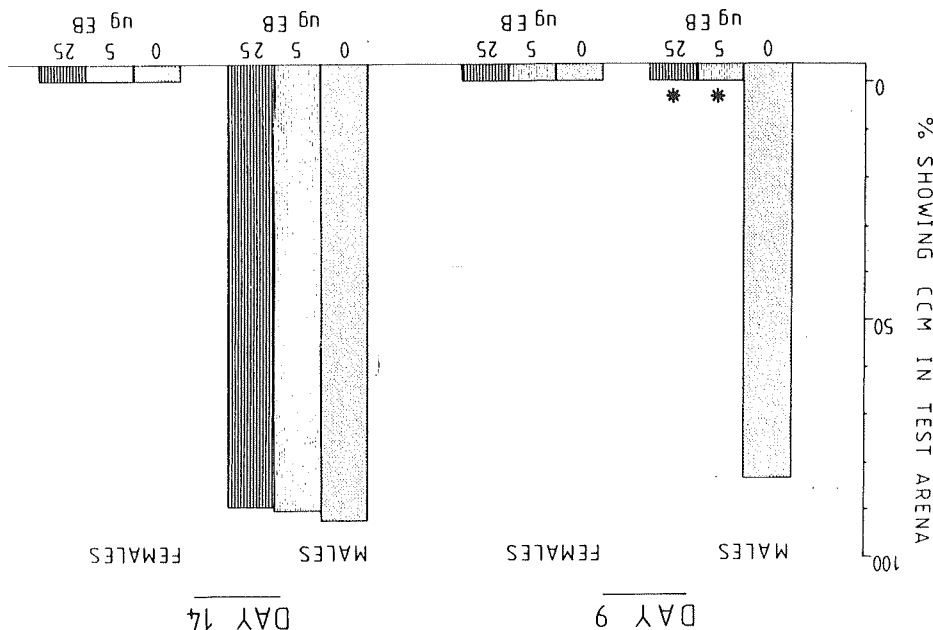
Taken together, these data satisfactorily explain why both TP and EB demasculinize behavior of quail embryos. Furthermore, in view of the very low enzymatic activity which is typical for the aromatase, they justify why high doses of T are necessary to demasculinize male embryos (only a small proportion of the molecules is transformed into active estrogens).

Another explanation for this requirement of unusually high doses of TP can be found in the brain metabolism of the hormone. In the bird brain, a very active 5 β -reductase transforms T into 5 β -dihydrotestosterone (5 β -DHT) and the corresponding diols which are almost completely devoid of androgenic properties.³⁷⁻⁴¹ Only activation effects of 5 β -androstanes have been tested and preliminary data suggest that they do not affect differentiation of sexual behavior; up to 2 mg of 5 β -DHT injected on day 9 of incubation does not reduce male copulatory behavior (J. Balhazart, unpublished results). We showed recently that this enzymatic activity is extremely high (at least 10 times adult values) in the hypothalamus of male and female quail throughout the incubation period and only declines progressively during the first weeks after hatching to reach adult values at the time of sexual maturation, i.e., around 4-6 weeks of age.^{42,43} Considering that 5 β -reductase transforms T into behaviorally inactive compounds which are in addition not aromatizable, it can be stated that this enzymatic activity protects embryonic males from being demasculinized by their endogenous T through its aromatization. This also probably explains why such high doses of TP have to be injected into the eggs to demasculinize male embryos.

THE CRITICAL PERIOD

Organizing effects of steroids are characterized by periods of maximal sensitivity, the so-called critical periods. In an attempt to determine the end of the critical period for male demasculinization, Adkins¹⁰ injected male embryos with 50 μ g EB on day 10, 11, 12, 13, or 14 of incubation or with an oil vehicle on day 10 as the control. Birds that had been treated before day 12 were significantly demasculinized, a marginal nonsignificant decrease in copulation was still observed in day 12 birds, and no effect at all was observed in birds injected on day 13 or 14. We have recently confirmed this result in our strain of quail using two different doses of EB (5 and 25 μ g) which were injected either on day 9 (active) or on day 14 (inactive) of incubation (M. Schumacher and J. Balhazart, unpublished data; see FIGURE 1). In this experiment, all birds were in addition gonadectomized 4 days after hatching to eliminate the confounding effects of postnatal steroids (see below). On the basis of this result (males cannot be demasculinized by exogenous EB after day 12), it was concluded that in quail the critical period for sexual differentiation ends on day 12 of incubation.^{2,10} This implies that the EB-induced demasculinization in males is also a reliable model for the demasculinization process by ovarian secretions in females. That this is not necessarily so was first suggested by a study of Hutchison⁴⁴ who showed that if female quail were ovariectomized in the first 12 hr after hatching, they were not completely demasculinized and a large proportion showed male copulatory behavior when treated with testosterone. This experiment, however, did not include females castrated as

FIGURE 1. Effects of treatment with estradiol benzoate (EB) during embryonic life on sexual behavior in male and female quail. Eggs were injected with 0, 5, or 25 μ g EB on day 9 or 14 of incubation; the young were gonadectomized 4 days after hatching and when adult received 60-mm Silastic implants of testosterone to activate behavior. CCM = cloacal contact movement. * = $2p < 0.05$, comparison with corresponding control (0 μ g) by Fisher tests.



adults and receiving the same testosterone treatment as a control so that it was not possible to ascertain whether the presence of male behavior in these neonatally gonadectomized females was due to test conditions or strain differences. The following experiments rejected the notion that sexual differentiation ends on day 12 of incubation.

In a first pilot study we confirmed the presence of copulatory behavior in females gonadectomized after hatching (1 week after in this case).⁴⁵ This again suggested that female demasculinization was not completed at hatching. In the next experiment,¹² we gonadectomized male and female quail at different times after hatching (from 1 day to 6 weeks) to examine the time course of this postnatal demasculinization (see FIGURE 2). Homotypical male behavior was consistently seen, following stimulation by T in adulthood, in the females which had been gonadectomized within 2 weeks post-hatch. It almost never occurred in females ovariectomized at 4-6 weeks after

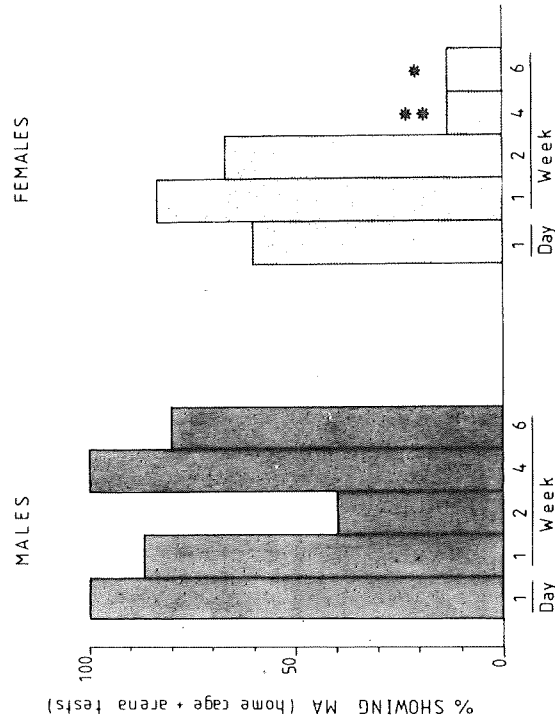
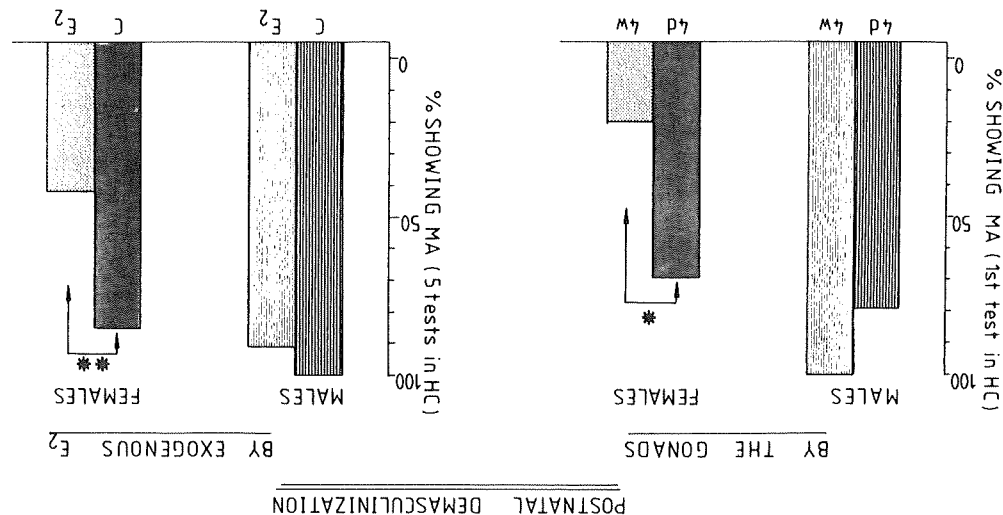


FIGURE 2. Effect of postnatal gonadectomy on sexual behavior. Birds were gonadectomized at different ages ranging from 1 day to 6 weeks post-hatch. When adult, sexual behavior was activated by 60-mm Silastic implants filled with T. Tests of sexual behavior were then performed both in the home cages and in a test arena. Pooled results are shown. MA = mount attempt. * = $p < 0.05$ and ** = $p < 0.01$, comparison with males of the same age by Fisher tests. (Modified from Schumacher and Balthazart.^{12,46})

hatching. In contrast, copulatory behavior was not affected by the age of castration in males and most birds in all groups showed vigorous male behavior. The lack of copulatory behavior in females castrated after 4 weeks of age could still have been attributed to the stress of the recent surgical operation. Another experiment was thus performed to test this hypothesis.⁴⁶ Males and females were gonadectomized at 4 days or 4 weeks of age but this time, all birds were operated at both ages (sham or real gonadectomy). This confirmed that females gonadectomized early in life are not completely demasculinized but that demasculinization is nearly completed at the age of 4 weeks. Once again, the age of castration had no effect on the behavior of males (see FIGURE 3).

FIGURE 3. Effects of steroids after hatching on female demasculinization. Left: birds were gonadectomized at 4 days (4d) or 4 weeks (4w) post-hatch and when adult received a 60-mm T implant to activate behavior. Percentage of birds showing mount attempt (MA) during the first test performed in the home cages (HC) is shown. Right: all birds were gonadectomized at 4 days post-hatch and implanted with Silastic capsules filled with E2 or left empty (C). Implants were removed after 1 month and behavior was activated by 60-mm T implants. Behavior (mount attempt, MA) was tested 5 times



This suggests that postnatal ovarian secretions are responsible for the complete demasculinization of female quail. We could then show that exogenous estradiol (E2) can mimic this effect of the ovary.⁴⁷ Male and female quail were gonadectomized at 4 days of age and immediately received E2-filled Silastic implants or empty implants as controls. These implants were removed at the age of 1 month and tests for copulatory behavior were then conducted with activation by exogenous T. Postnatal E2 treatment significantly reduced the sexual behavior in neonatally gonadectomized females but was without effect in males (see FIGURE 3, right). The postnatal sensitivity to the organizing action of E2 is thus sexually differentiated before hatching as females but not males (in agreement with previous data: see Reference 10 and FIGURE 1 in this paper) are demasculinized by exogenous E2.

A final series of experiments also established that while low doses of E2 can demasculinize females during their first month of life, even high amounts of hormone are without effect in adult birds (males and females). This suggested that the estrogen target tissues do not maintain their sensitivity to the organizing effect of E2 throughout life.

Although neonatally ovariectomized females consistently show homotypical male behavior if treated with T, it must be clearly stated that they are already partly demasculinized. This is mainly supported by the following facts: (1) their sexual behavior is less vigorous than that of males: they rarely show cloacal contact movements (full copulation) and their behavior is usually limited to neck grabs and mount attempts (see FIGURE 4); and (2) they show male behavior under optimal conditions: their sexual activity is, for example, very weak in the test arena that we normally use to test the behavior of males, but it becomes comparable to that of males if tests are performed in the home cages. The latter observations extend to birds a question recently raised by Feder⁴⁸ in the discussion of differentiation in rodents. Do steroids really organize the behavioral capacity of the animals or do they determine under what social or environmental conditions the behavior will be displayed (see also References 48-50)?

SEXUAL DIFFERENTIATION AS A BIPHASIC PROCESS

In quail, the information about the sensitive period during which E2 organizes male sexual behavior can be summarized as follows:

- (1) Exogenous E2 demasculinizes male embryos up to day 12 of incubation and is not effective thereafter.
- (2) Females gonadectomized soon after hatching retain partly the capacity to show male behavior.
- (3) Demasculinization of females is completed during the first month of life under the influence of ovarian secretions. The sensitivity to the organizing effect of E2 is lost in adult females.
- (4) Exogenous E2 demasculinizes neonatally ovariectomized females but is without effect in neonatally castrated males.

One set of data thus points to a brief sensitive period ending on day 12 of incubation and another set of data to a continuous process of female demasculinization which is only completed between 2 and 4 weeks post-hatch. To reconcile these data, we propose that sexual differentiation is a biphasic process. A limited "period of sensitization" corresponding to the classical critical period (ending on incubation day 12) would be followed by a continuous "period of organization" which would end only when birds

approach puberty (see FIGURE 5). A short hormonal stimulus during the period of sensitization would change irreversibly the sensitivity of nervous tissues to the organizing effects of gonadal steroids. Once the sensitization effect takes place, the process of differentiation continues for an additional period of time, as in female quail. This model takes into account all the data available to date and also explains a number of paradoxical findings related to rodent sexual differentiation.

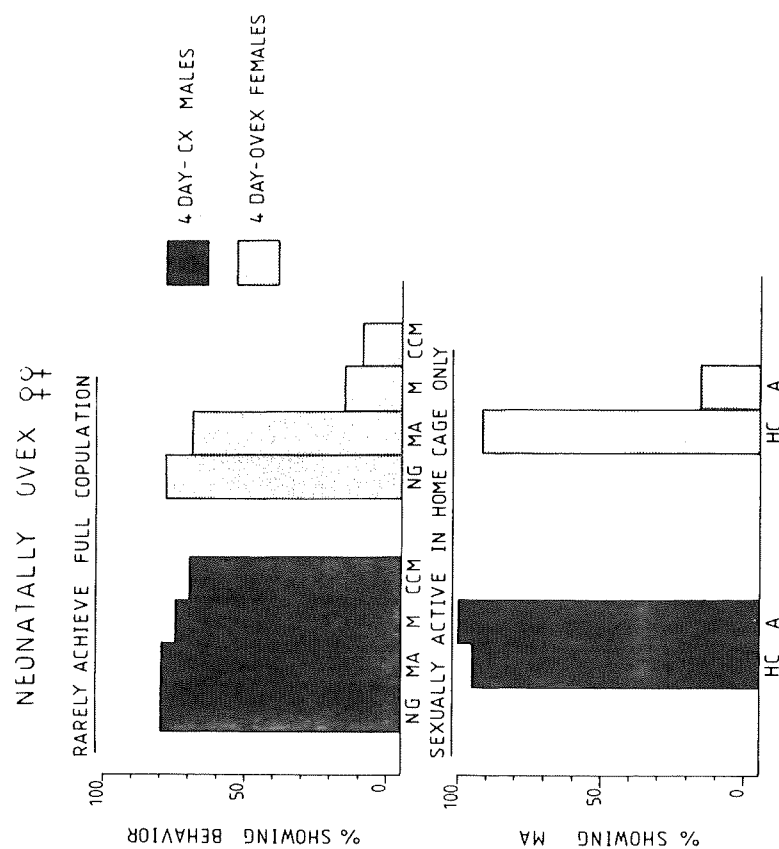
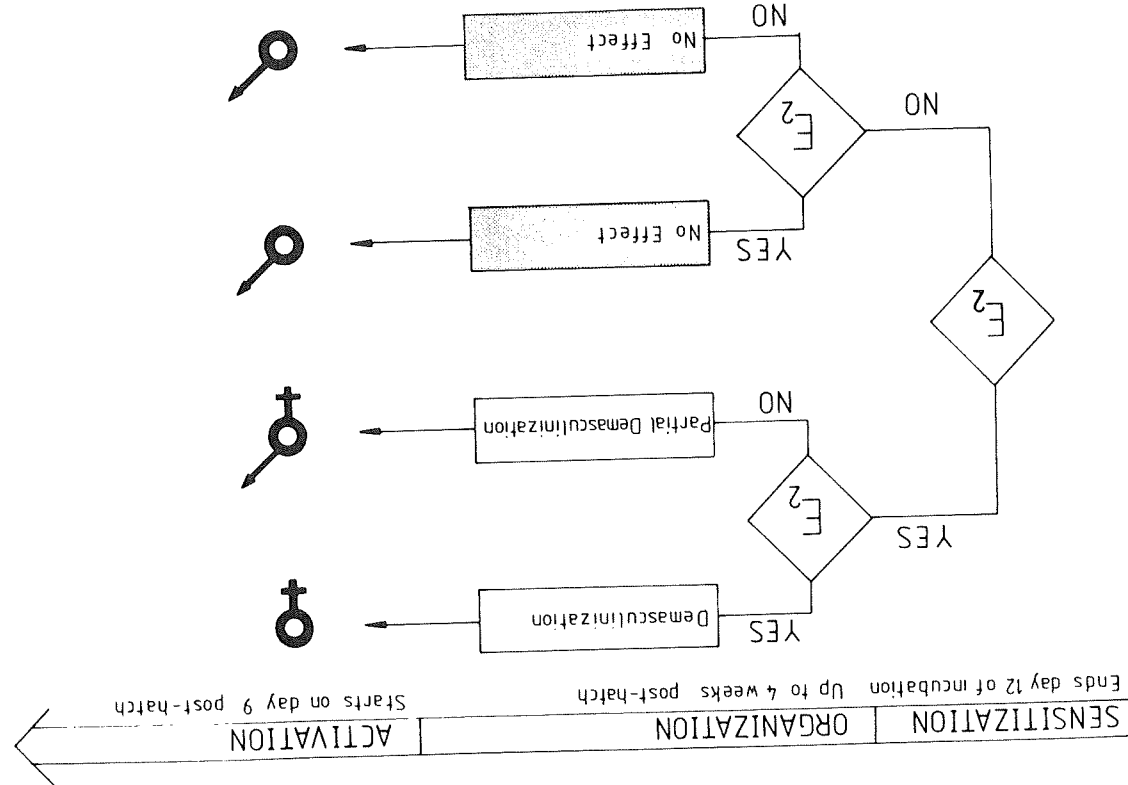


FIGURE 4. Neonatally ovariectomized (OVEX) females are already partly demasculinized. Top: their sexual behavior rarely includes mount (M) and cloacal contact movement (CCM) and is mainly limited to neck grab (NG) and mount attempt (MA). Results are relative to the first test performed in the home cages for a group of neonatally gonadectomized birds whose behavior was activated by 60-mm T implants. Bottom: they are active mainly in their home cage (HC) and show little activity in the test arena (A). Data concern the same birds as above tested six times in the home cages and three times in the arena.

In rats, sexual differentiation results from the exposure of males to T during the perinatal period (from a few days before birth to 5 days after). The lordosis response is lost (defeminization) and male behavior is reinforced (masculinization) if young rats are exposed to T during that period.¹ This model based on castration and replacement therapy experiments is, however, poorly supported by radioimmunoassays of plasma levels of T in young males and females.⁵¹ Male rats have higher androgen

FIGURE 5. Model of the two-step sexual differentiation in quail. To obtain full demasculinization, estradiol (E2) must be present in the animals during a period preceding day 12 of incubation (period of sensitization) and during the post-hatching life (period of organization). If E2 is not present during the sensitization, it is no longer active later in life.



levels than females only during a short period of development (around day 18 post-conception) and after that androgen levels almost completely overlap in both sexes (even though mean levels are always a little higher in males). The sexual differentiation thus cannot result from a prolonged exposure to a different hormonal milieu which suggested to Weisz and Ward⁵¹ that differentiation of sexual behavior involves a sequence of two androgen-sensitive phases. High plasma testosterone on days 18-19 postconception sensitizes the fetal brain of males to the organizing action of androgens circulating in lower concentration at later stages of development. These data represent circumstantial correlative evidence suggesting that the model proposed here for quail development could have a more general applicability.

Experimental data also support this notion. Female rats show a cyclic pattern of luteinizing hormone (LH) secretion and in addition adequate treatment with E2 and progesterone (P) stimulates a dramatic LH surge. Males by contrast show a more tonic LH release and do not show a clear surge of LH in response to E2 and P.^{52,53} This dimorphism is the result of androgen action on the brain during the first 5 days after birth. Androgen injections after the age of 6 days do not markedly alter the pattern of LH secretion in females. If females are lightly androgenized (with subthreshold doses of TP) on day 5 of life, they will display normal estrous cycles in adulthood (LH secretion is not defeminized). However, after a few cycles, they will lose this capacity and become anovulatory.⁵⁴⁻⁵⁶ This delayed anovulatory syndrome (DAS) results from the exposure to postpubertal ovarian steroids as shown by ovariectomy experiments and is not observed if the females are not androgenized postnatally.⁵⁷ These experiments demonstrate that brief hormonal stimulation during the neonatal period in rats can alter for an extended period the sensitivity to steroids.⁵⁸ These data in the rat provide an example of the differentiation model that we propose here for quail. Future research should demonstrate if such a biphasic mechanism is a widespread phenomenon in vertebrate differentiation and what is its physiological basis. On the basis of current data, several possibilities can be considered:

- (1) Steroid action during the sensitization phase causes survival of specific neurons which will differentiate during the organization period.⁵⁹
 - (2) The sensitizing action of steroids induces in target cells steroid receptors or steroid metabolizing enzymes (e.g., aromatase) which may be required for the subsequent organizing action of steroids.⁵¹
 - (3) During the sensitization phase, E2 alters in a permanent way the conformation of specific genes or produces long-term accumulation of specific mRNA which would be translated later during the second exposure to the organizing hormone. Interestingly, it has already been suggested that the estrogen-induced vitellogenin synthesis in *Xenopus laevis* and in hens may involve such mechanisms: a primary hormonal stimulation enhances the effectiveness of a secondary exposure to estrogens.⁶⁰⁻⁶²
- Experiments are now in progress with quail to collect additional data supporting this two-step differentiation model and to elucidate its physiological basis.

ISSUES AND DIRECTIONS FOR FUTURE WORK

Do Periods for Activation and Organization of Behavior Partly Overlap in Quail?

The above data clearly show that female demasculinization is not completed before 2 weeks post-hatch and that males cannot be demasculinized by exogenous E2 after

day 12 of incubation. It is then interesting to note that male-type sexual behavior can be activated in young castrated males with 20-mm Silastic T implants during the first 2 weeks post-hatch (first positive tests on day 9 with implants placed on day 7 but no earlier age was tested; M. Schumacher and J. Balthazart, unpublished data). In this experiment, no sexual behavior was observed in T-treated females which supports the notion that they are partly demasculinized at hatching and thus probably have a higher threshold for behavior activation.

Separate experiments have, however, demonstrated that T or DHT Silastic implants can stimulate crowing in both male and female quail chicks (first 3 weeks of life) with equal efficiency (same frequencies and latencies of occurrence were noted and dose-response curves were similar in both sexes⁶³) and it has been clearly established that the hormonal induction of crowing in adult quail is sexually dimorphic.^{67,11} Thus in quail, organization and activation of male sexual behavior partly overlap in time as previously shown for female sexual behavior in the rat^{64,65} (see also the paper by C. L. Williams in this volume). The concept of independent periods for these two processes should thus be reconsidered and experimental work should be undertaken to understand their precise specificity (e.g., do the same hormonal stimuli in young animals activate and at the same time differentiate the behavior?).

Do We Have an Adequate Terminology for Differentiation?

Comparison of quail and rat studies on differentiation also suggests a few reflections about the taxonomy of differentiation processes and the use of this terminology in the literature. Four independent processes can be distinguished on the basis of the presence-absence of the considered behavior in the "anohormonal" condition and of the differentiating effects of steroids. Homotypical male behavior can be present in the anohormonal condition and lost after exposure to steroids, a process called demasculinization, which apparently explains differentiation of copulatory behavior in quail. Alternatively, male behavior can be acquired (or increased) under the influence of steroids (it is absent in anohormonal conditions). Examples of this masculinization process can be found in the differentiation of copulation in rodents⁶⁶ and in the acquisition of song by zebra finches.^{2,67,68}

Mirror images of these processes can be defined for differentiation of homotypical female behavior patterns. The lordosis response of rodents is differentiated by a defeminization of the males; lordosis is present in the anohormonal state and lost following exposure of males to testosterone. It is more difficult to find examples of the feminization process, that is, the acquisition following exposure to steroids of a female behavior pattern which is absent in the anohormonal condition (see, however, Reference 69 for a possible feminization of the lordosis response in female rats during sexual maturation).

On the basis of these logical definitions, several remarks can be made. First, confusion exists in the common use of terms. The loss of male behavior in male quail following TP or EB treatment is frequently referred to as feminization, and similarly the maintenance of male behavior in females treated in the egg with antiestrogens⁶⁴ has been called masculinization when it should be absence of the demasculinization. A similar confusion exists in the literature concerning rodents. This sloppiness in terminology not only complicates communication between scientists but it also conceals important theoretical problems.

Are We Asking All Pertinent Questions?

A complete description of sexual differentiation in a single species should include the study of the organization of both male and female homotypical behaviors, the identification of their status in anohormonal conditions, and description of the effects of steroids at different times in the ontogeny. This task has not yet been achieved in any species.

In quail, the best-known avian model, the only differentiation process which has received detailed attention is demasculinization. Female behavior is reputed to be isomorphic (it can be elicited in both males and females with EB injection) and no study has thus been made on the role of steroids on its organization. This is unfortunate for two reasons. First, it is not certain that because the behavior is present in both sexes, it does not require at some stages of development the organizing action of steroid hormones. Second, it is quite possible that female behavior, although present in both sexes, is quantitatively differentiated. All activation experiments performed so far used large doses of EB (50 µg; see References 6 and 9) which probably produce in males supraphysiological plasma levels of estrogens (in females 50 µg EB raises plasma E2 to 1215 pg/ml while levels in intact birds are around 100-200 pg/ml; see References 2 and 70). Sexual differences in the threshold for activation of squatting are thus quite possible. On the other hand, male behavior is supposed to be present in the anohormonal state and is differentiated only by demasculinization of the females. What is really the evidence for the absence of a hormonal requirement in the organization of homotypical male behavior? It is actually very limited. We know that the behavior is normally organized in birds castrated just after hatching (there is thus no postnatal masculinization⁴⁶) and in embryos treated on day 9 of incubation with the antiestrogen, CI-628,²⁴ which presumably means that the presence of estrogens is not required for its organization. No experimental data can, however, be found to reject the idea that male behavior requires for its normal development the presence during the ontogeny of physiological levels of testosterone (it would be masculinized).

The same argument can be developed for the organization of lordosis in rodents. The lordosis response is supposedly present in the anohormonal state and is differentiated by a specific loss of the behavior (defeminization) in males following exposure to testosterone (acting via neural aromatization⁶⁶). Once again what is the evidence that no hormonal stimulus (estrogens) is required for the normal development of the homotypical behavior? Some experiments suggest that low levels of androgens around the time of birth potentiate feminine as well as masculine behavior in female rodents (see references in MacLusky and Naftolin⁶⁶). Many studies have demonstrated the morphogenetic effect of E2 on brain structures (organization of the sexually dimorphic nucleus of the preoptic area⁷¹; growth of dendritic processes⁷²). The importance of estradiol to neural development clearly challenges the notion that the female neural and behavioral phenotype merely results from the absence of androgen exposure.^{73,74} The effects of steroids on *in vitro* differentiation of neuronal cells clearly suggest that no pattern of differentiation is intrinsic to the nervous tissue, but rather that both male and female patterns require organization by steroids.

Should We Use the Concept of Anohormonal Development?

At the behavioral level, evidence for the anohormonal development of a given behavior is always limited with respect to the hormone concerned (there is no need for T in the normal development of lordosis in rats, no need for E2 in the development

of copulation in quail) and with respect to the period of development studied (testicular secretions play no role in the postnatal differentiation of copulation in quails but what about before hatching; is the organization of lordosis really independent of estrogens during the embryonic stages⁷⁵ or even during juvenile life⁶⁹). The absence of a hormonal requirement is probably impossible to demonstrate because an embryo without hormone is impossible to obtain and would not be viable. The notion of anohormonal or neutral development should thus be used cautiously by making reference to the hormone and the period of development concerned (i.e., behavior develops normally without this hormone during that period). At the same time, experimental work should refine our knowledge on hormonal requirements in the behavioral organization in order to go beyond the simplistic view that sexual differentiation mainly proceeds by loss in one sex of behavioral characteristics which develop in the other sex without any hormonal control.

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Reciprocity in Neuroendocrine and Evolutionary Approaches to the Study of Reproductive Behavior

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Behavioral neuroendocrinologists must deal with a lot of complexity within the endocrine systems underlying reproductive behavior. Some reasons for this complexity are the existence of numerous hormones which affect reproductive behavior, hormonal interactions within individuals, and quantitative hormonal differences between individuals and species. The object of this paper is to point out that a full understanding of such physiological complexity can expand our appreciation of the evolutionary process, and, conversely, that an evolutionary perspective can provide an understanding of these complex physiological mechanisms.

Rosenblatt and Komisaruk¹ have written that "Daniel S. Lehrman's lifelong love and study of animal behavior gave us a wealth of new insights into reproductive behavior and evolution." In creating the Institute of Animal Behavior of Rutgers University, Dr. Lehrman provided an environment in which physiological and evolutionary perspectives on behavior could, to use his own words, "intersect" and "compliment" each other.

We behavioral neuroendocrinologists accept as given, and are now fairly adept at handling, complex mechanisms underlying behavior; this was not always so. In the 1960s our approach, a reasonable one given our knowledge of psychoneuroendocrinology at that time, was to look for a simple neural button that would generate mating postures if "pushed" and for the steroid hormone that "pushed" it. In fact, for rat lordotic behavior, it appeared likely that the neural button was in the hypothalamus and that the steroid hormone, estrogen, triggered it. Of course, many investigators, including Drs. Lehrman and Rosenblatt, recognized that hormone-behavior relationships were affected by social and other environmental factors, but within an organism, we looked for simple mechanisms. Nevertheless, a complicated picture quickly emerged even in the "simple system" underlying rat lordosis, and it became clear that it was incorrect to look for one hormone that serves as a "button pusher." Our work at the Institute² illustrated that lordosis is elicited by multisensory stimulation in the absence of a steroid hormone of ovarian or adrenal origin, that either estrogen or progesterone alone potentiates it, and that both hormones acting together have a synergistic effect. Since then, prostaglandins,^{3,4} luteinizing hormone-releasing hormone,^{5,6} and adrenocorticotrophic hormones⁷ have also been implicated in the mechanism underlying lordosis.

Now that we recognize that many hormones may act to potentiate a single behavioral response, how do we deal with the multiplicity? We consider hormones one