

Hormonal regulation of brain circuits mediating male sexual behavior in birds

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Abstract

Male sexual behavior in both field and laboratory settings has been studied in birds since the 19th century. Birds are valuable for the investigation of the neuroendocrine mechanisms of sexual behavior, because their behavior can be studied in the context of a large amount of field data, well-defined neural circuits related to reproductive behavior have been described, and the avian neuroendocrine system exhibits many examples of marked plasticity. As is the case in other taxa, male sexual behavior in birds can be usefully divided into an appetitive phase consisting of variable behaviors (typically searching and courtship) that allow an individual to converge on a functional outcome, copulation (consummatory phase). Based primarily on experimental studies in ring doves and Japanese quail, it has been shown that testosterone of gonadal origin plays an important role in the activation of both of these aspects of male sexual behavior. Furthermore, the conversion of androgens, such as testosterone, in the brain to estrogens, such as 17β -estradiol, is essential for the full expression of male-typical behaviors. The localization of sex steroid receptors and the enzyme aromatase in the brain, along with lesion, hormone implant and immediate early gene expression studies, has identified many neural sites related to the control of male behavior. The preoptic area (POA) is a key site for the integration of sensory inputs and the initiation of motor outputs. Furthermore, prominent connections between the POA and the periaqueductal gray (PAG) form a node that is regulated by steroid hormones, receive sensory inputs and send efferent projections to the brainstem and spinal cord that activate male sexual behaviors. The sensory inputs regulating avian male sexual responses, in contrast to most mammalian species, are primarily visual and auditory, so a future challenge will be to identify how these senses impinge on the POA–PAG circuit. Similarly, most avian species do not have an intromittent organ, so the projections from the POA–PAG to the brainstem and spinal cord that control sexual reflexes will be of particular interest to contrast with the well characterized rodent system. With this knowledge, general principles about the organization of male sexual circuits can be elucidated, and comparative studies relating known species variation in avian male sexual behaviors to variation in neural systems can be pursued.

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1. Introduction

1.1. Why focus on birds? A traditional model taxa for the study of hormone–behavior interrelationships

This paper will review current knowledge about the neural and endocrine bases of male sexual behaviors in birds, with a particular focus on the role of gonadal sex steroid

hormones. Given the current trend in the life sciences toward a focus on a few model species to elucidate molecular and cellular mechanisms of physiology and behavior, it may seem surprising to have a review that considers only avian species. One might suggest that such a review would be useful for historical reasons; what is arguably the first experimental study in endocrinology, conducted by Berthold [52], concerned an avian species, the domestic fowl, and included endpoints such as courtship behaviors. In the more modern era, studies of birds have often played a key role in our understanding of neuroendocrine mechanisms regulating reproductive behavior, stemming in part from the seminal

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studies of Lehrman et al. conducted at Rutgers University on ring doves (*Streptopelia risoria*) that were initiated in the 1950s (e.g., Refs. [110,111]). It is not just for historical reasons, though, that it is useful to review the neuroendocrine basis of male sexual responses in birds. The advantages that first attracted behavioral neuroscientists and neuroethologists to birds are still apparent, and research ranging from the cellular level to the systems level of analysis in relation to male sexual responses continues to be conducted (e.g., Refs. [9,16,25,26]).

What are the advantages of studying birds? Birds are generally diurnal and rely on visual and auditory cues for most of their social interactions. There are 9000 to 10,000 extant species today, and they range across habitats and continents. This combination of abundance and conspicuousness makes them easy to study. There is therefore a relatively large amount known about bird behavior both in the field and in the laboratory. This wealth of data allows avian behavioral neuroscientists to place their work in a natural context and to use information about the natural history to guide experimental investigations into mechanisms. Birds often possess well-defined brain nuclei that can be directly related to behavior. The avian song control system is perhaps the best known example, but the neural circuit regulating male sexual behavior also exhibits similar properties [13,131]. Knowledge about these well-defined nuclei involved in male sexual responding can guide cellular and molecular studies.

The avian brain also exhibits a remarkable degree of hormone-induced or hormone-related neuroplasticity that makes avian species attractive for neuroendocrine study. This phenomenon is perhaps best documented in the avian song control system, in which Nottebohm [125] discovered dramatic seasonal changes in male canaries in the volume of key telencephalic nuclei that control courtship song. Such seasonal variation in brain area volume was later found to involve changes in cell number in some cases, such as nucleus HVC of the nidopallium (abbreviation now used as a proper name, previously misnamed hyperstriatum ventrale, pars caudale) [173]. How can changes in neuron number occur in the adult brain? Nottebohm et al. [85,126] also discovered that adult birds, unlike mammalian species, have widespread ongoing neurogenesis in the telencephalon. Gonadal steroid hormones, which play such an important role in controlling the volume changes, do not seem to regulate the rate of neurogenesis but rather the probability that a new neuron will be incorporated into a functional neural circuit that is a steroid hormone target site [55,108]. Morphological brain plasticity is not limited to the song system of oscines. Studies in other species have shown, for example, that the volume of the sexually dimorphic medial preoptic nucleus in quail varies as a function of the endocrine state of the bird [133]. Plasticity in this case does not, however, rely on neurogenesis and neuronal death but rather reflects changes in neuronal size and spacing [131,133].

Hormone-related plasticity in avian neuroendocrine systems is not limited to obvious brain changes in morphology. Proteins involved in steroid hormone action in the avian brain also exhibit some unusual and useful features, again if compared with mammalian species. For example, the key steroid metabolizing enzyme aromatase (estrogen synthase) exhibits a remarkably high degree of enzymatic activity in the avian brain (10–20 times higher in preoptic area–hypothalamus of various avian species than in rats; see Refs. [144,159]), which makes its detection by enzymatic assays or immunohistochemical methods relatively easy [31,82,151]. In some species, these high levels of activity are not limited to the diencephalon but are also detected in the telencephalon [148,149,176]. In the diencephalon of quail and doves the enzymatic activity is highly regulated by testosterone (T), making investigations of avian species especially useful for studies of the cellular basis of enzymatic regulation [26,151,166]. Plasticity in reproductive systems in birds is not just limited to the brain. In seasonally breeding species, the testis changes observed in birds are of far greater magnitude than those generally observed in mammals. In birds, such changes are several hundredfold between the breeding and non-breeding season, while in a commonly studied seasonally breeding mammal, the Syrian hamster, such changes are three- to fivefold in magnitude [66]. This difference in the magnitude of seasonal variation is associated with qualitative differences in the underlying cellular mechanism. In European starlings (*Sturnus vulgaris*), seasonal regression of the testis involves apoptosis of Sertoli cells, a cell type not known to undergo cell death in mammalian species [189].

One important question to ask is whether this high degree of plasticity observed in avian neuroendocrine systems related to male sexual behavior and reproduction is of purely parochial interest. If such phenomena are restricted to birds, their value to investigators working on other taxa would be limited. This does not seem to be the case. First, studies of avian neuroanatomy indicate that organization and chemical neuroanatomy of the avian brain is far closer to mammalian brain architecture than previously thought [138]. Also, in many instances, seemingly surprising discoveries first made in avian species have subsequently been discovered to occur in mammalian systems, just at a lower degree of magnitude, making them more difficult to detect. For example, adult neurogenesis does occur in the hippocampus and perhaps cortex in monkeys, but it is harder to detect and not as prominent as that observed in the avian brain [87]. Another example of such continuity is that the enzyme aromatase is regulated in the mammalian diencephalon in a manner quite similar to what has been seen in birds; it was just not as easy to detect, and it remains a more challenging phenomenon to study in these species [142,143]. Most evidence points to the fact that studies of avian species are of general significance.

In this review, we will first discuss some basics of behavioral aspects of male sexual behavior, then move on to

the sensory and hormonal control of this behavior, and end with a review of current knowledge about neural circuits that underlie these behaviors in male birds.

2. Basic description of male sexual behavior in birds

Thinking about the organization of male sexual behavior in birds, as is the case in other taxa, has been influenced by ethological concepts such as the fixed-action pattern and the distinction between appetitive and consummatory behaviors first articulated by pioneer ethologists such as Tinbergen [171], Lorenz [114] and Baerends [14]. Avian male sexual displays represent some of the paradigmatic examples of fixed-action patterns [11], which are stereotyped, species-typical behavior patterns. The appetitive/consummatory distinction was adopted to explain systematic variation in the occurrence and frequency of certain behaviors. Some species-typical behaviors result in a functional outcome that is associated with a reduction in motivation (consummatory responses), while other more variable behaviors allow an individual to converge on this functional outcome (appetitive responses [170]). This sort of dichotomy is potentially problematic when one tries to apply it to complex sequences of behavior. For example, is the entire copulatory sequence the consummatory response or just the cloacal contact movements and associated sperm transfer? Because of ambiguities such as this, the concept should be used cautiously, but the distinction continues to be useful to both ethologists and experimental psychologists in the elucidation of neuroendocrine mechanisms mediating a range of behaviors [170]. The study of sexual behavior is an area where this distinction has been particularly useful in guiding mechanistic studies. Beach [49] formally introduced this idea to the field of behavioral endocrinology by pointing out its usefulness to the analysis of male sexual behavior based on his studies of rodents. More recently, this same paradigm has been applied usefully to studies of sexual behavior in females (e.g., Ref. [134]).

In males, appetitive sexual behavior consists of searching for and approaching a potential mate, whereas the consummatory component includes the actual contact between the sexes culminating in copulation [49]. Appetitive male sexual behaviors include most courtship behaviors that function to attract females and stimulate them to bring them into a sexually receptive condition. In birds, males are known to exhibit a wide diversity of visual and vocal displays that function in this manner [11]. As expected there is a large amount of species variability. In some cases, stereotyped movements involving elaborate plumage displays are used to attract and stimulate females. In other cases, vocal behavior is paramount, and in many cases there is a combination of these two sorts of displays. What is clear is that communication involving other sensory modalities such as olfaction or touch is not particularly important. Experimental paradigms for the investigation of appetitive

male sexual behavior have been developed in domesticated species such as the Japanese quail (*Coturnix japonica*). It was discovered that when a male quail copulates with a female for a single time in one area, there is a marked change in his behavior. After copulating with a female, the male will stand in front of a window that provides him with visual access to the female for most of the day [68,69]. This is a robust response that is easily quantifiable and provides a useful way to investigate the neuroendocrine mechanisms regulating male appetitive sexual behavior [23,25,36].

Consummatory male sexual behavior consists of the copulatory act itself. This requires that males mount females so that gamete transfer can be facilitated. In most species, gamete transfer involves what is sometimes referred to as the “cloacal kiss”. These are cloacal contact movements that facilitate the deposition of sperm in the female. There are often other stereotyped motor patterns that precede cloacal contact movements per se. For example, in Japanese quail, copulation consists of a sequence of stereotyped movements progressing from neck grab to mounts and cloacal contact movements [4,106]. In species with a penis-like intromittent organ, the copulatory act involves intromission as well. Most copulations between members of a pair are solicited by either the male or the female member of the pair [53]. In a comparative study of 213 Palearctic bird species, it was found that females were more apt to solicit copulations than males, indicating that in most of these species, females control pair copulations [53]. However, males also play an important role in initiating copulations in many species, as was described above for Japanese quail (though see Ref. [70]). In species in which female copulation solicitation is common, male neck-grabbing behavior prior to copulation is uncommon [53]. Precopulatory and copulatory behavior is also often associated with the production of specific calls again that can be uttered by either the male or the female.

3. Sensory control of male sexual responses

Male sexual responses are controlled by the interrelationship between physical cues in the environment and social cues. One of the best-studied physical cues is variation in day length or photoperiod. The importance of photoperiod is most apparent in temperate zone species that breed seasonally in the spring when food resources are most abundant and likely to support the successful production of as many healthy offspring as possible. At the proximal level, this abundance of food is very often best predicted by the vernal increase in photoperiod, and many species are therefore photoperiodic: The activity of their hypothalamo-pituitary-gonadal axis is stimulated by long days and decreased in short days. In addition, some species, especially those living in harsh environments at high latitudes, terminate breeding long before the photoperiod reaches its maximal value at the summer solstice to ensure that enough time will be left in favorable condition to raise young that will be able to

migrate before winter. These birds display a phenomenon called photorefractoriness: After exposure to a given number of long days, their hypothalamo-pituitary–gonadal axis shuts off, even though photoperiod is still long (see Ref. [66]).

Other species, living mostly at lower (tropical) latitudes, do not synchronize their reproduction with the changes in photoperiod [93]. Birds living in desert environments will, for example, breed when vegetation growth is stimulated by episodic rain (e.g., the zebra finch [128]). Other species that inhabit the tropical forest will have extended periods of reproduction eventually centered on seasons when their specific food is the most abundant. This strategy presumably maximizes the use of food resources that are never present in extremely large amounts but are available for a long duration. The perception of daylength needed to regulate seasonal cycles in birds does not involve the eyes, but rather deep brain extra-retinal receptors (see Refs. [66,79,129] for reviews). The definitive identification of this receptor is still not complete, though several lines of evidence point to cells in either the lateral septum or the infundibulum [146].

Social cues also have a powerful effect on endocrine physiology, and these effects seem to be mediated primarily by visual and auditory systems [15]. In a selected number of avian species, it has been experimentally demonstrated that the development of the reproductive system is also modulated by social signals from the congeners, in particular, from conspecific females that represent potential mates (see Ref. [16] for a review). This observation obviously influences the expression of male sexual behavior and its timing during the year. These data will not be reviewed here, since they have been reviewed recently in detail [16,17,186]. On a shorter time scale, social (sexual) signals (presence of the female) are also needed for the elicitation of male sexual responses. The nature of these sexual signals and the sensory pathways involved will be reviewed briefly.

3.1. The nature of the social stimuli that elicit male sexual responses

Experimental analyses of the sensory bases of the effects of one sex on the endocrine physiology of the other have been conducted in the most detail in ring doves. Original studies were conducted in females for pragmatic reasons: Sensitive assays for plasma hormone were not available, and the growth of the ovarian follicle in females provided a very rapid and sensitive measure of the effects of the social interaction. Friedman [83] designed an ingenious experimental apparatus in which sounds and images originating in a central cage housing a male were made available in different combinations (due to transparent or nontransparent partitions, one-way mirrors, and loud-speakers) to different females located in adjacent cages. In this set up, one female could, for example, see and hear the male courting her, another one could hear the male and see him courting the

first female (courtship was thus not oriented toward her), another one could see but not hear him, another one could hear but not see him, etc. This experiment showed that a combination of visual and acoustic stimuli is required for optimal stimulation of ovarian development. Interestingly, spatial congruence of visual and acoustic inputs was important in determining the level of responding, suggesting that the female is actually “reading” the partner’s message (see Ref. [56] for further discussion).

Soon thereafter, radioimmunoassay of steroid hormones became more widely available and could be used to assess the endocrine effects of the exposure to auditory and visual sexual stimuli. A male dove taken from isolation will immediately display male-typical courtship behaviors such as the bow coo when presented with an intact female dove. This behavioral response is followed by a significant rise in plasma T levels within 30 min [80,127]. This rise is attenuated if the male is paired with an ovariectomized female who does not exhibit sexual displays [127]. Additional experiments identified the sensory modalities underlying this physiological reaction. If males were separated from females by a clear panel so that they could see and hear the female but not have tactile interactions with her, they had hormone levels slightly but not significantly lower than those of males who could fully interact with the female. Furthermore, if the male was deafened, he did not exhibit a rise in T, and his hormonal levels were close to those of isolated males (see Refs. [56,127] for review; Fig. 1).

Similarly, the female’s endocrine and behavioral responses to the male are dependent on both visual and acoustic cues emanating from the male (see Ref. [56] for review). Interestingly, the female’s behavioral response to the male contributes to her own endocrine development by means of a self-stimulation mechanism [57,58]. The predominance of visual and auditory stimuli in the control of reproduction was best characterized in ring doves, in particular females, and olfactory or tactile sensations seem

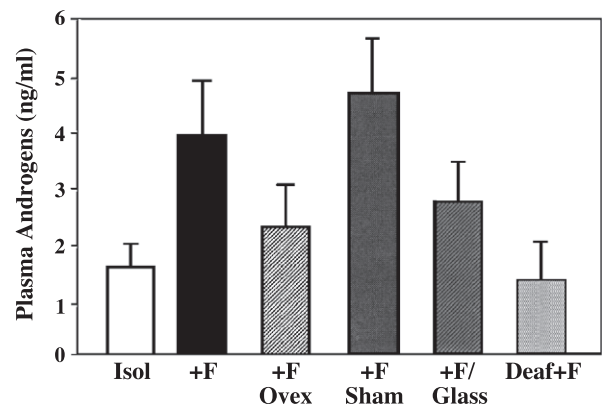


Fig. 1. Mean (\pm S.E.M.) plasma androgen concentrations in male ring doves 24 h after they were paired with a female (+F), an ovariectomized female (+F Ovex), a sham-operated female (+F Sham), a female placed behind a glass partition (+F/Glass) or in deaf males exposed to a normal female (Deaf+F). Control males were kept in isolation (Isol). Redrawn from data in Ref. [127].

to play only a minor role. The available information suggests that this conclusion can be applied to a wide variety of avian species.

In quail, Guyomarc'h [91] also demonstrated that ovarian development in females can be stimulated by exposure to male vocalizations. In male quail, as is true of many Galliform species, visual cues appear to be of primary importance in the control of social interactions and seem to be sufficient to elicit copulatory responses in a male treated with high levels of T. The presentation of a female model has been shown to elicit the male copulatory response in turkeys, chicken, and quail (see Ref. [71] for discussion). In addition, Domjan and Nash [71] have demonstrated that static visual cues, as obtained from a taxidermically prepared model, are sufficient to induce social proximity behavior and suppress crowing in male quail in the absence of behavioral, auditory, and olfactory cues. The behavior induced by the female model was primarily elicited by visual cues from the head and neck region, which are sexually dimorphic in quail [71]. Additional stimuli, especially vocalizations and behavioral cues such as copulation solicitation, will enhance the response and probably play a role in the natural situation. Olfactory cues seem of little importance, although a few studies may call for a re-evaluation of this widely accepted idea.

In mammals, the olfactory information that plays a role in the activation of sexual behavior in males and females reaches the POA through a pathway that includes the cortico-medial amygdala and the bed nucleus of the stria terminalis (BSTM) [116,152]. In vivo tract-tracing has revealed the presence in quail of an important projection from the arcopallium and in particular the nucleus taeniae of the amygdala to the medial preoptic nucleus [22], suggesting that olfactory inputs could reach the medial preoptic nucleus of the quail. The importance of olfactory information in the control of behavior in birds is usually considered to be minimal, but a limited number of studies indicate the chemical information originating in the female could modulate some aspects of the reproductive behavior (ducks [37,107]; auklets [92]; see also Ref. [179]). The projection from nucleus taeniae to the medial POA would then acquire a particular importance.

4. Hormonal basis of male sexual responses in birds

In most species of birds that have been investigated, male sexual responses are under control of testosterone. This dependence of avian reproductive behaviors on testicular secretions was in fact demonstrated long ago when Berthold showed that castration abolishes the behavioral manifestations of sexual maturity in domestic cocks and that effects of castration could be reversed by a testicular graft [52], an experiment considered as the first classical experiment in behavioral endocrinology. More sophisticated experiments have since confirmed and extended this finding to a variety

of experimental models (e.g., Refs. [20,48,123]), and, with the advent of sensitive radioimmunoassays, it has become possible to correlate circulating levels of T with the behavior exhibited by birds in the laboratory as well as in the field [180,182,184,186]. These studies identified a great diversity of patterns in the annual/seasonal changes in plasma levels of T in different species of birds as a function of their reproductive strategies and have also confirmed the key role played by this steroid in the control of reproduction.

Two main types of evidence must be collected to ensure that a given behavior is under the control of a specific hormone. Suppression of the hormone source should inhibit the behavior, and the behavior and hormone titer in the blood should co-vary in time. Experiments of these two types are now available to demonstrate the prominent role of T on male sexual behavior in a variety of avian species and are briefly reviewed below.

4.1. Correlations between changes in plasma T and male sexual responses

It has been known for many decades that the recrudescence of male reproductive activities in the spring is associated with the increase in testicular size. In male birds, the seasonal changes in testes size and histology were correlated with the occurrence of reproductive behavior in a variety of species from many orders, such as songbirds (great tit [158]; white crowned sparrow [181]; pied flycatcher [157]; European starling [168]), ducks (mallard [100]; common eider [86]) or columbidae (feral pigeon [120]; wood pigeon [113]). Similar evidence has also been collected in laboratory conditions in which transfer from short to long days induces the testicular development and expression of male sexual behavior (e.g., quail [145]).

The advent of radioimmunoassays in the late 1960s and early 1970s allowed repeated measures of plasma sex steroids so that these correlations could be markedly refined. For example, during an annual cycle we followed the changes in plasma T levels (one blood sample every 2–4 weeks) and in a variety of reproductive behaviors in a group of male domestic ducks of the Rouen breed [33]. This revealed a high correlation between changes in plasma T levels as assessed by radioimmunoassay and in the frequencies of sexual behaviors *sensu stricto* (mounts and copulations; Fig. 2). The annual peak in social displays, a form of courtship behavior, occurred before the maximal levels of plasma T were reached, indicating that either these behaviors were maximally activated by low levels of this androgen or their activation depends at least in part on other hormones (see Refs. [28,33] for discussion).

Many studies of this type have been performed during the past 20 years, in particular in small passerine species, but also in other bird orders studied in their natural environment. This has permitted the description of the seasonal changes in plasma T in species that differ by the number of broods they raise in a year, their degree of

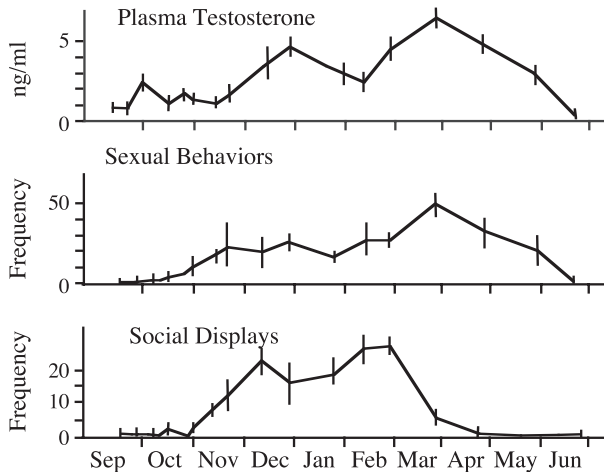


Fig. 2. Annual variations (means \pm S.E.M.) in plasma T and in the frequency of male sexual behaviors and social displays in a group of captive male ducks. Redrawn from data in Ref. [33].

parental investment, the amount of aggressive interactions they show with their congeners or their socio-sexual system (e.g., monogamous, polyandrous and polygynous species). Specific relationships have therefore been identified between these variables. This topic has been the subject of many published reviews (e.g., Refs. [99,180,183–185]) and is beyond the scope of the present paper.

4.2. Experimental manipulations of plasma T

4.2.1. Hormone removal and replacement

A large number of studies have now established that the removal of the testes abolishes, or at least markedly depresses, the expression of all aspects of male sexual behaviors while these behaviors are restored with a treatment with exogenous T. This is the case in cockerels [65,72], Japanese quail [6,50], wild and domestic ducks [75,135], gulls [124], zebra finches [12,137], pigeons [74] and ring doves [8,59] (see Fig. 3 for an example in quail).

It must be noted that not all responses in the behavioral repertoire of birds depend to the same extent on the presence of high concentrations of T. Singing, which can be considered as a form of appetitive sexual behavior displayed to attract the female, is markedly decreased by castration and enhanced by T in zebra finches [94] but less so in starlings. Singing in starlings is not restricted to the spring when plasma T are elevated but also occurs at high rates when plasma levels of T are basal and often undetectable [73]. Recent work indicates that different forms of singing can be distinguished as a function of the social context in which they appear, and their endocrine control seems to be quite different. Singing of male starlings directed at females (as observed mostly in the spring) is clearly androgen-dependent and largely disappears following castration, but non-directed singing is maintained at normal levels in castrates and is unaffected by T treatments [136] (see Ref. [18] for a more detailed discussion of this topic).

Similarly, a number of avian species display year-round territorial behavior and aggression. The fact that these birds remain aggressive in the fall, when circulating levels of androgens are extremely low, indicates that their aggressive behavior does not directly depend on testicular androgens [163,185]. Similar findings have been reported in tropical birds that also display aggressive behavior during most of the year in a manner that is not correlated with the changes in plasma T levels [95,96]. Surprisingly, these behaviors are inhibited by treatments aimed at decreasing androgen and/or estrogen action (e.g., antiandrogens and aromatase inhibitors), suggesting that they are activated by sex steroids even if plasma T levels are low [160–162]. The source and nature of these steroids remain partially unclear at present, but a contribution of sex steroids that would be directly synthesized in the brain appears to be very likely [164,165].

Although there are clear links between the activation of male sexual behavior and plasma concentration of androgens, some potential exceptions have been noted in the literature. For example, castrated male white-crowned sparrows have been shown to engage in mounting behavior in a manner indistinguishable from that of intact [118]. In this case, the efficacy of the castration was confirmed by radioimmunoassay measurements. These have not been followed up carefully but do suggest that male sexual

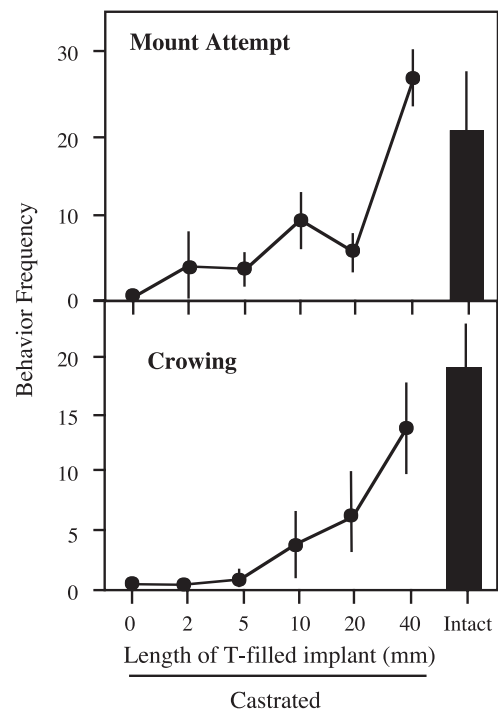


Fig. 3. Effect of castration and exposure to increasing doses of T on male sexual behavior (mount attempts) and on crowing occurrence frequencies in male Japanese quail. Castration completely suppressed the expression of both types of behaviors, and they were restored to the level observed in gonadally intact birds by the highest dose of T. Castrates were treated with Silastic subcutaneous capsules of various lengths (2–40 mm) filled with crystalline T. Redrawn from data in Ref. [32].

behavior can occur to varying degrees independently of the action of T.

4.2.2. Endocrine specificity

It was originally thought that androgens such as T activate male-typical sexual behaviors (mostly in males), while estrogens and progesterone were responsible for the activation of female typical behavior (mostly in females). It soon became clear that the situation is not so simple. As early as 1949, Guhl [88] showed that estrogen injections can activate male copulatory movements in castrated cockerels. This observation and similar findings in mammals eventually led to the notion that somehow T must be transformed in the brain into an estrogen before it produces its behavioral effects (see Ref. [24], this volume). Many studies indeed identified metabolic pathways that catalyze T into a variety of behaviorally active or inactive metabolites (see Ref. [21] for review).

Three major pathways deserve special consideration in connection with the activation of male sexual behavior in birds. On the one hand, aromatase leads to the production of estrogens (estradiol, E2 or estrone, E1), and 5 α -reductase produces the potent androgen, 5 α -dihydrotestosterone (5 α -DHT). These two steroids play a significant role in the activation of specific aspects of the behavioral repertoire. On the other hand, 5 β -reductase leads to the formation of the behaviorally inactive 5 β -dihydrotestosterone (5 β -DHT). 5 β -DHT is apparently devoid of any behavioral activity and seems unable to activate any aspect of the male repertoire of reproductive behavior in adult birds (see, however, Refs. [20,34,35] for apparently conflicting data in young chicks and their discussion). These transformations are thermodynamically irreversible, at least in physiological conditions. The activity of these three enzymes is regulated in a manner that appears to be specific to the species considered, the sex of the subjects and the anatomical location considered (see Ref. [20,21] for review). More important for the purpose of the present review, many studies have demonstrated in a variety of avian species that E2 and 5 α -DHT, alone or in combination, mimic most, if not all, behavioral effects of T, suggesting that at the cellular level, T effects are due to the action of these metabolites. Evidence supporting this statement has been reviewed extensively in several papers [16,20,21] and will just be illustrated here by two sets of studies on avian species commonly used in the laboratory, the Japanese quail and zebra finch (see Fig. 4).

In both species, castration drastically reduces the percentage of males displaying sexual behavior (quail) or courtship behavior including singing (finches). These behaviors are restored by a systemic treatment with exogenous T or the aromatizable androgen, androstenedione (AE). A similar restoration is also induced by treatments that provide both an estrogen (estradiol benzoate, EB in Fig. 4) and an androgen such as 5 α -DHT (or its propionate ester DHTP). These metabolites in isolation are usually less efficient, although in quail, EB alone causes a full activation

of copulatory behavior, implying a major role of aromatization in the activation of this behavior [5,7,94] (see Ref. [24], this volume).

Aromatization of T also seems to be critical for the activation of appetitive sexual behavior, at least in quail. Most studies on avian sexual behavior were until recently confined to the analysis of the controls of consummatory sexual behavior. In quail, the social proximity response mentioned previously in this paper (a male will stand in front of a window providing a view of a female after he has copulated once in the adjacent arena) has proven to be a valuable tool in the study of the hormonal control of appetitive response. A series of experiments clearly established that this social proximity response can be activated in castrated male quail by a treatment with exogenous estrogens and is blocked in T-treated castrates by daily injections of an aromatase inhibitor [27,36]. The production by males of rhythmic cloacal sphincter movements in response to the view of a sexually mature female, another form of appetitive sexual behavior in quail [153,154], is also inhibited by aromatase blockers in male quail [167]. Together, these data thus support the notion that activation of appetitive sexual behavior in quail also depends on T aromatization.

Treatments providing androgens only (androgenic compounds that cannot be aromatized such as 5 α -DHT, methyltestosterone [MT] or fluorotestosterone [FT]) usually activate little or no sexual behavior (see Fig. 4). However, pure androgens are able by themselves to activate other aspects of the behavioral repertoire such as crowing and strutting in quail [3] or bow cooing (a display with a major aggressive component usually seen at the beginning of the reproductive cycle) in ring doves [8]. As mentioned above, 5 β -DHT is behaviorally ineffective.

4.2.3. Site of steroid action

The brain, and in particular the medial POA (see below), is the major site of hormone action necessary for the expression of sexual behaviors, and most of this section will be devoted to a review of the evidence supporting this conclusion. It must be kept in mind, however, that hormones have widespread effects in the entire organism, and there are at least three ways that hormones act on peripheral tissues that are relevant to behavior control: by changing effector muscular structures, changing social signals or affecting sensory inputs to the brain (see Refs. [16,20,98] for detailed discussion).

Because sexual behavior is activated by T in birds as in mammals (see above), the localization of androgen receptors in the brain was first used as a guide to identify the brain regions where the steroid action could be implicated in behavior control. Autoradiographic studies as well as immunocytochemistry and in situ hybridization concur to indicate that androgen binding sites in the avian brain are located, like in other vertebrates, mainly in the POA–hypothalamus, in a few other limbic regions (nucleus striata terminalis, nucleus taeniae) and in the mesencephalic

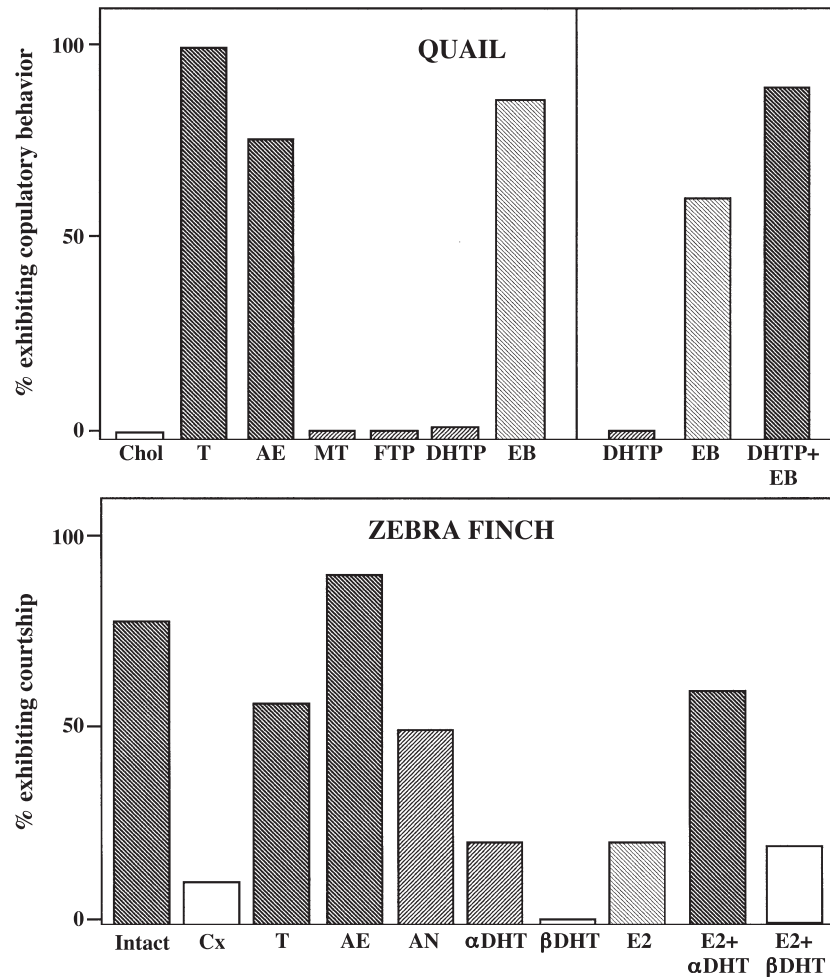


Fig. 4. Percentage of castrated male quail that copulated (top) and percentage of castrated (Cx) male zebra finches that displayed courtship behavior (bottom) following treatment with various steroids that provided androgenic stimulation (cross bars from top right to bottom left), estrogenic stimulation (cross bars from top left to bottom right) or both (cross-hatching). Chol: cholesterol, T: testosterone; AE: androstenedione; MT: 17 α -methyltestosterone; FTP: 6 α -fluorotestosterone; DHTP: 5 α -dihydrotestosterone propionate; EB: estradiol benzoate; Intact: gonadally intact subjects; AN: androsterone; α DHT: 5 α -dihydrotestosterone; β DHT: 5 β -dihydrotestosterone; E2: estradiol. Redrawn from data in Ref. [5] (top left), [7] (top right) and [94] (bottom).

nucleus intercollicularis (see Ref. [16] for review). In ring doves, it has been shown that androgen receptor expression is highly regulated in the brain of males during the reproductive cycle, being most prevalent during the period of courtship when males are engaging in sexual behavior and much lower during the parental phase [51,109]. Songbirds also possess androgen-sensitive nuclei that are part of the song control system, but this topic is outside the scope of the present review (see Ref. [147,150] for reviews).

In the 1960s, the first experiments based on the stereotaxic implantation of steroids in the brain indicated that, in mammals, the POA is the most important of these androgen-binding sites for the activation of male sexual behavior [112]. Experimental studies performed soon thereafter confirmed that in birds also, the medial part of the POA plays a key role in the activation by steroids of male copulatory behavior. It was found that both in castrated ring doves and cockerels, T implants restore a full copulatory behavior when placed in the medial POA but not at other

brain sites [44,45,104,105]. Similar results were later obtained in additional species such as the Japanese quail [177,178].

A sexually dimorphic nucleus was later identified in the quail POA [175]. The medial preoptic nucleus (POM) in this species is larger in males, which show copulatory behavior in response to T, than in females, which do not [4,38]. Furthermore, the volume of the POM regresses after castration and is restored to values observed in sexually mature males by a two week treatment with T [132,133], suggesting that this structure is involved in the activation of male copulatory behavior. Stereotaxic experiments directly confirmed that the POM specifically is a necessary and sufficient site of T action for the activation of copulatory behavior in males. Electrolytic lesions of the POM, but not of the surrounding POA, completely suppress copulatory behavior activated in castrated males by Silastic implants containing T [41]. Conversely, stereotaxic implants filled with T activate all aspects of copulatory behavior in

castrated males if their tip is located within the cytoarchitectonic boundaries of the POM but not if it is located in the adjacent POA [41,42]. These data demonstrate that T action in the POM is sufficient to activate copulatory behavior in adult male quail, but it must be stressed that they do not rule out an action of this steroid at additional sites in the central nervous system (see below).

Other studies in quail demonstrated that T must be aromatized within the POM in order to exert its behavioral effects [40,178]. Stereotaxic implants of aromatase inhibitors in the medial preoptic region significantly inhibit the activation of copulatory behavior produced by a systemic treatment of castrated males with T [30,40,178].

4.2.4. Aromatase expressing cells as markers of the circuit controlling male sexual behavior

The aromatase dependence of behavior was also used to further refine the analysis of the anatomical sites of steroid action on quail copulatory behavior. Aromatase-immunoreactive (ARO-ir) neurons are a specific marker of the sexually dimorphic POM [31,43], and the number of these immunoreactive cells is markedly increased by a systemic treatment with T [43]. Aromatase immunocytochemistry was thus used to map the areas that are destroyed by electrolytic lesions or stimulated by the stereotaxic implantation of T in the POA, and these measures were correlated with the behavior of the animals to identify the parts of the POA that are critical in the activation of copulatory behavior [42].

Electrolytic lesions of the POA disrupted the activation of male sexual behavior by T only if they destroyed a significant part of the POM identified by ARO-ir neurons, and the disruption of behavior was correlated with the decrease in the number of ARO-ir neurons. Conversely, stereotaxic T implants in or close to POM activated sexual behavior and increased the number of ARO-ir cells in the nucleus [42].

Correlative analyses suggested that a part of the POM just rostral to the anterior commissure is critical for the activation of copulatory behavior. The best correlation between the behavioral deficits induced by electrolytic lesions and the size of the lesions was indeed in this area. In addition, a high correlation was observed between the behavior activated by T implants and the number of ARO-ir cells that were induced by T in this area rostral to the anterior commissure [42].

We also used the approach response called “learned social proximity response” by Domjan et al. [68,69] to analyze the neural circuit that may be involved in the control of appetitive sexual behavior in quail. Castrated male quail chronically treated with T were first trained during eight sessions to acquire the social proximity response. They were then submitted to bilateral electrolytic lesions aimed at the POM and retested during nine additional sessions for the presence of appetitive and consummatory sexual behavior [23]. The POM lesion was

then reconstructed in sections where ARO-ir cells had been stained by immunocytochemistry.

Lesions affecting the POM ARO-ir cells completely abolished consummatory sexual behavior, but they also significantly decreased all measures of appetitive sexual behavior. Interestingly, the two components of male sexual behavior were affected the most by lesions of different subregions of the POM. Damage to a portion of the POM just rostral to the anterior commissure resulted in the complete inhibition of consummatory sexual behavior (as already discussed above), while damage to a more rostral part of the POM selectively inhibited appetitive sexual behavior [23]. Lesions of the POM also markedly depressed the expression of rhythmic cloacal sphincter movements produced by males in response to the view of a sexually mature female [23,153,154]. Therefore, these data strongly suggest that the preoptic region plays an important role in the regulation of motivational as well as performance aspects of male sexual behavior.

Interestingly, recent studies carried out in starlings indicate that the amount of singing produced by males in a reproductive context (presence of a female and of nest material) is also controlled by the medial part of the POA [140,141]. Singing is used to defend a territory and/or to attract females and can therefore be also considered as a form of appetitive sexual behavior. This finding thus reinforces the result of studies carried out in quail demonstrating that the POA is implicated in the control of appetitive as well as consummatory sexual behavior.

Immunocytochemical studies had also shown that other regions besides the POM contain dense populations of ARO-ir cells. Two major groups have been identified: one running from the dorso-lateral aspects of the ventromedial nucleus (VMN) to the infundibular area of the hypothalamus and one overlapping with the medial part of the BSTM [31,82]. A smaller population of ARO-ir cells is also located in the nucleus taeniae of the amygdala [82], an area of the avian forebrain that is the homologue to components of the amygdala as defined in mammals [169,190]. Stereotaxic lesion and implantation studies were therefore carried out to test whether some of these brain areas could be implicated in the activation of male sexual behavior.

In mammals, the VMN plays a key role in the activation by steroids of female sexual behavior [46], and, based on the few data available, the VMN should play a similar role in birds [84]. The infundibular area seems to be implicated in the control of gonadotrophin secretion [63,64]. These brain regions and the aromatase-expressing cells they contain thus do not seem to be directly involved in the control of male sexual behavior, but no experiment has to our knowledge directly tested this idea.

In contrast, data from stereotaxic experiments based on electrolytic lesions and T implants suggest that the aromatase cells in the BSTM could play a role in the activation of some aspects of male reproductive behavior. Bilateral electrolytic lesions were placed in the BSTM of T-

treated castrated males that had previously acquired the learned social proximity response indicative of appetitive male sexual behavior, and all subjects were re-tested after the lesion [23]. Lesions aimed at the BSTM had no effect on appetitive sexual behavior, as measured by the expression of the social proximity response, but significantly decreased consummatory sexual behavior ($\pm 40\%$ inhibition). However, in a parallel study, T-implants directed at the BSTM failed to activate significant amounts of consummatory or appetitive (social proximity response and rhythmic cloacal sphincter movements) sexual behaviors in castrated male quail [139]. These data thus suggest that the BSTM could play a role in the activation by steroid of male behavior, but additional work should be carried out to fully qualify this conclusion.

Previous work has also shown that relatively large bilateral lesions to the nucleus taeniae of the amygdala attenuate measures of male sexual behavior [169]. In particular, bilateral ablation of taeniae decreased the speed at which males approached a female, the time they spent in association with her, the number of cloacal contact movements they engaged in during a 5-min interaction with a female and finally the number of cloacal sphincter muscle movements displayed in the presence of a female.

These data thus suggested that the ARO-ir cells present in nucleus taeniae could play a role in the control of male sexual behavior, and therefore, we investigated the effects of discrete lesions restricted to nucleus taeniae and to an adjacent area (arcopallium intermedium, AI) on the expression of appetitive (learned social proximity response; rhythmic cloacal sphincter movements) and consummatory (mount attempts and cloacal contact movements) aspects of male sexual behavior [1]. Lesions confined to nucleus taeniae and to AI did not influence the acquisition nor the maintenance of the two responses indicative of appetitive sexual behavior. In contrast, lesions of nucleus taeniae significantly increased the occurrence frequencies of mount attempts and cloacal contact movements when administered before the beginning of behavior testing and increased the frequency of mount attempts only when performed on sexually experienced subjects. No effect of AI lesions could be detected. The discrepancy between these results and previous experiments in quail may reflect procedural differences but are more probably due to differences in locations of the lesions, which were restricted in the current study to the anterior part of taeniae, whereas those in the Thompson et al. study were in the posterior part of this nucleus. These findings indicate that there is a larger degree of functional heterogeneity in the nucleus taeniae than previously thought. The effects of lesions also suggest that this nucleus, like the medial amygdala in mammals, may be involved in the regulation of both appetitive and consummatory aspects and in the control of sexual satiety as previously suggested in mammals [76,77,187,188].

In summary, the aromatization of T into an estrogen plays a critical role in the activation of appetitive and consumma-

tory aspects of male sexual behavior in birds as exemplified by quail. Although ARO-ir cells are present in several brain areas, it seems that ARO-ir neurons located in the POA are those that are most critical for behavioral activation. A possible contribution of ARO-ir cells in the BSTM and in nucleus taeniae of the amygdala is also suggested but should be further investigated before any firm conclusion can be reached.

5. Neural circuit mediating male sexual behavior

As just reviewed, several types of functional neuroanatomical investigations have guided us to brain sites that might be involved in the neural control of avian male sexual behavior. However, this circuit is still only partially understood. We know the most about core diencephalic and mesencephalic pathways that are involved in the activation of both appetitive and consummatory sexual responses. Based on the lesion studies, hormone implant work and the chemical neuroanatomical studies of hormone receptors, the POA obviously is a key site for the integration of information involved in the regulation of male sexual responding. Therefore, most neuroanatomical studies completed to date have focused on this nucleus. It is also apparent from many studies that the POA sends a prominent projection to the periaqueductal gray (PAG; also referred to by its Latin name, *substantia grisea centralis* in many avian atlases) in birds as well as in other vertebrate taxa and that this projection from the POA to the PAG forms the core of this circuit (e.g., Refs. [2,119]). The full complement of sensory inputs and motor outputs to the POA–PAG connection involved in the control of male sexual behavior still needs to be elucidated. We will focus on two approaches to the study of the avian male sexual circuit in this section: studies using immediate early gene expression to identify brain areas involved in sexual responses and tract-tracing studies of the afferent and efferent connections of the POA–PAG connection.

Studies of the expression of the immediate early gene *c-fos* have been helpful in birds just as they have in mammalian species [16]. For example, in quail, copulation induces the appearance of Fos immunoreactive cells in the POA, the ventral mesopallium, parts of the arcopallium and the nucleus intercollicularis [115]. Induction of Fos was observed throughout the rostral to caudal extent of the preoptic region of males from the level of the tractus septomesencephalicus to the level of the anterior commissure, and in the rostral part of the hypothalamus to the level of the supraoptic decussation. It is unlikely that the Fos induction in males observed in this study resulted from copulation-induced endocrine changes, because copulation did not affect plasma levels of luteinizing hormone or T. Rather, the responses were due to copulation-associated somatosensory inputs and/or to stimuli originating from the female. These responses are roughly similar to what has

been described in similar studies in rats [47,61]. The comparison is of interest, given that the male birds do not have an intromittent organ and do not rely on olfactory stimuli to detect females; therefore, these responses are not specific to somatosensory stimuli from the penis or to olfactory stimuli from the primary or accessory olfactory systems.

The induction of Fos was also observed in several brain areas such as the ventral mesopallium, medial arcopallium and bed nucleus striae terminalis in males that were allowed to express appetitive sexual behavior (watching a female through a narrow window) but were not allowed to copulate [172]. The data therefore clearly indicate that the immediate early gene expression is not related solely to the control of the copulatory act but also to the processing in a variety of telencephalic association areas of stimuli originating from the female.

Another immediate early gene has also been studied in relation to copulatory behavior in quail, namely ZENK (the avian homologue of *egr-1*). This gene was first identified in birds, because it is induced in males and females at high rates in the auditory telencephalon in response to conspecific vocalizations [117]. Studies in quail found that copulatory activity markedly increased the number of ZENK-ir cells in the bed nucleus striae terminalis, both in the subdivision dorsal to the anterior commissure and at a more caudal level at which this structure forms a characteristic “V” shape [19]. Increases in ZENK-ir cell numbers observed in this area after expression of appetitive sexual behavior were not statistically significant. By contrast, ZENK-ir cell numbers in the nucleus intercollicularis increased in males in all groups placed in the test chamber. These data indicate that induction of the ZENK protein occurs in behavioral contexts related to reproductive behavior; whether this results only from the performance of these behaviors or whether this induction is related to components of these behaviors that involve learning and reward, as has been suggested in other species, is however unknown.

5.1. Tract-tracing studies illustrating the connectivity of the POM and BST

Tract-tracing studies are an essential approach needed to identify the afferents and efferents of the POM. Studies completed in quail involving the *in vitro*, lipophilic fluorescent dioctadecyl-tetramethyl-indocarbocyanine (DiI) demonstrated a number of bi-directional connections between the POM and several hypothalamic and thalamic nuclei [29]. DiI implantation in aldehyde-fixed tissue demonstrated anterograde projections from the POM to the tuberal hypothalamus, the ventral tegmental area (VTA) and the PAG. Dense networks of fluorescent fibers were also seen in several hypothalamic nuclei, such as the anterior medialis hypothalami, the paraventricularis magnocellularis and the ventromedialis hypothalami. A major

projection in the dorsal direction was also observed from the POM toward the nucleus septalis lateralis and medialis.

Fluorescent cells were seen in all these areas, demonstrating that the POM receives afferent projections from all these regions. Implantation of DiI into the PAG also revealed massive bidirectional connections with a large number of more caudal mesencephalic and pontine structures. The PAG therefore appears to be an important center connecting anterior levels of the brain to brainstem nuclei that may be involved in the control of male copulatory behavior. Most of these bidirectional projections could be confirmed by implanting DiI in the identified targets of POM and observing fluorescent label in POM (cells or fibers) [29]. After implantation of DiI in the POM, fluorescent fibers but no fluorescent cells were seen in the nucleus intercollicularis (ICo), suggesting the presence of a unidirectional pathway connecting the POM to ICo. However, no experiment has so far confirmed the existence of this connection by applying a retrograde tracer in ICo.

In vitro tracing with DiI is not optimal for the identification of long distance projections. Therefore, additional tracing studies were completed with cholera toxin B-subunit (CTB) or with red fluorescent latex beads in order to obtain more information on more distant connections of the POM and of the other group of aromatase-immunoreactive cells centered on the BST [22]. This technique confirmed all the connections of the POM that had been identified with DiI. Retrogradely labelled cells were observed in the telencephalic areas (hippocampus, septum, arcopallium), hypothalamus (many areas in periventricular position), thalamus and mesencephalon and pons. CTB tracing confirmed that most of these connections are bidirectional. In addition, a strong input from the rostral part of the nucleus taeniae of the amygdala to the POM was identified. Furthermore, large numbers of retrogradely labelled cells were found in the major catecholaminergic cell groups including dopaminergic areas such as the retrorubral field, substantia nigra (SN), VTA and noradrenergic cell groups such as the locus coeruleus and subcoeruleus (see below).

A significant number of brain areas were identified that appear to project both to the POM and the BST. There were quantitative differences between these projections. As compared with the POM, injections of tracer in the BST labeled a smaller number of neurons in the septal area and in a periventricular position throughout the rostro-caudal extent of the hypothalamus. Many neurons in the ventromedial nucleus of the hypothalamus were, for example, filled with tracer after injection in the POM, but this nucleus was completely devoid of retrogradely transported tracer after injection in the BST. In contrast, injections of tracer in the BST labeled more cells in the nidopallium, arcopallium, VTA, SN, locus coeruleus and subcoeruleus region than injections in the POM [22].

Taken together, these data indicate that a large number of brain areas are connected to the POM and/or activated during the expression of appetitive or consummatory

aspects of male sexual behavior as revealed by an increased expression of immediate early genes (see above) or by an increased glucose accumulation [39,67] (see Fig. 5). These areas are therefore likely to be involved in the activation of behavior. None of these areas receive, to our knowledge, direct afferent inputs coming from the eyes or ears, but these stimuli could affect behavior by indirect pathways.

The social life of quail, like many other avian species, is organized mainly by visual and acoustic cues, whereas olfactory and tactile stimuli are usually considered to play only a minor role [97,111]. Based on the tracing studies carried out so far, two types of sensory information should be able to reach the POM, a steroid-sensitive structure that plays a key role in the integration and activation of male sexual behavior.

The projection from the nucleus dorso-lateralis anterior thalami (DLA; geniculate complex) to POM appears to have a particular relevance for the processing of visual informa-

tion. The DLA is a part of the geniculate complex that receives direct retinal input [54,89,90] and contains a high density of melatonin receptors [62,130]. Therefore, primary visual information could potentially reach the POM through this pathway. Moreover, the cerebrospinal fluid (CSF)—contacting neurons of the lateral septal organ and of the tuberal region are frequently considered to be extraretinal photoreceptors [155,156]. These two regions send dense inputs to the POM and these connections could also relay information about the environmental level of light to the dimorphic nucleus. Information about environmental light can therefore reach the POM through different routes (retinal input through DLA, deep photoreceptors through septal region and tuberal hypothalamus).

We do not know at present whether specific visual information about the mating partner reaches the dimorphic nucleus. The thalamo-fugal [89,90,101] or retino-thalamo-hyperstriatal [54] pathway is connected to POM via nucleus

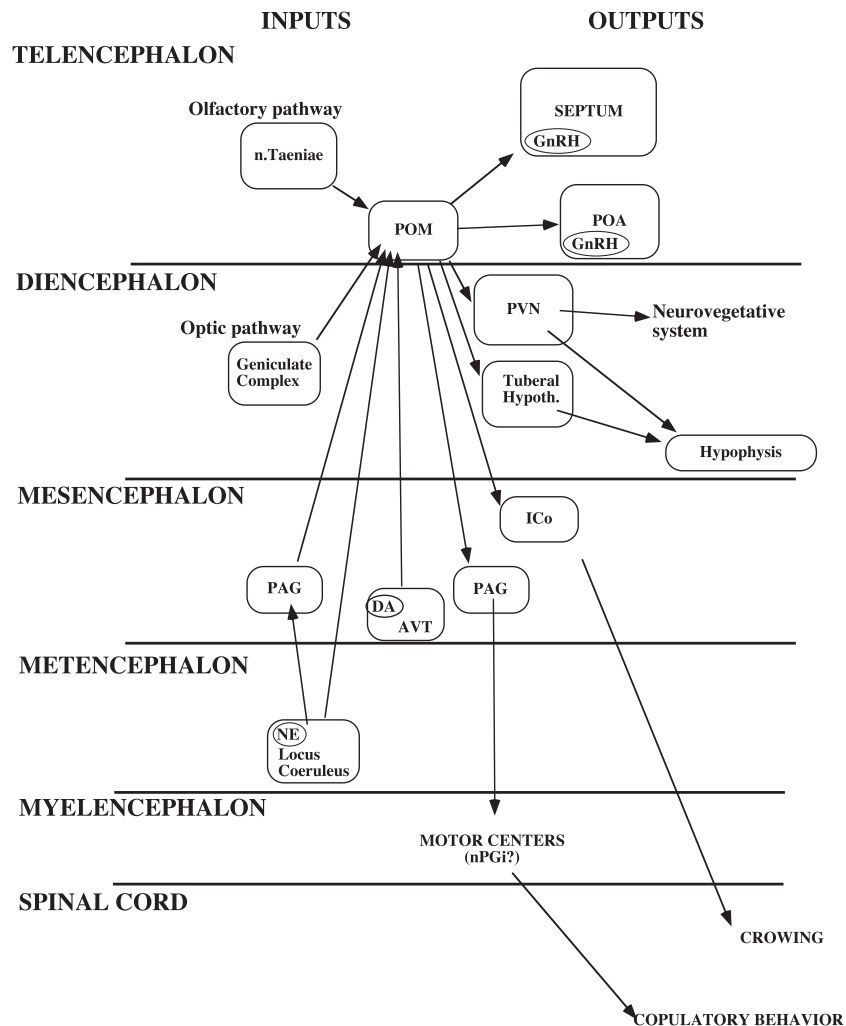


Fig. 5. Schematic representation of the neural circuit mediating male sexual behavior in birds based essentially on studies carried out in birds with special emphasis on the inputs and outputs of the medial preoptic nucleus (POM). The figure illustrates the putative visual and olfactory inputs to the circuit and the outputs to nuclei potentially mediating the expression of reproductive behaviors (copulation) and vocalizations (crowing). AVT: ventral tegmental area; DA: dopamine; GnRH: gonadotropin-releasing hormone; ICo: intercollicular nucleus; NE: norepinephrine; nPGi: nucleus paragigantocellularis; PAG: periaqueductal gray; POA: preoptic area; PVN: paraventricular nucleus of the hypothalamus. Modified from Ref. [131].

DLA, but it is unlikely that the level of integration of the visual information in the geniculate complex (DLA) would permit the identification of the female as such. Projections to the POM originating from telencephalic regions where the final processing of visual information takes place would be required for that purpose. A diffuse projection from the hyperpallium accessorium (part of the visual Wulst) to the POM has been identified by retrograde tracing with cholera toxin or with fluorescent microspheres [22]. This connection could convey complex visual information; however, more functional studies on the visual Wulst would be needed [89,90], and a better definition of its connection to the POM should be established by specific tract-tracing experiments, before the significance of this anatomical relation can be established.

No direct auditory input to POM of quail has been so far identified, but it is useful to note that visual cues provided by a taxidermic female model are sufficient to stimulate the appetitive and consummatory aspects of sexual behavior of male quail [71]. It is therefore plausible that auditory stimuli are not implicated in the direct control of male sexual behavior in this species. Cheng et al. [60] have, however, identified cells in the preoptic region of ring doves that exhibit auditory responsiveness, especially in association with the presentation of the female's own nest coos.

In mammals, olfactory information derived mainly from the accessory olfactory system reaches the POA and appears to play a key role in the activation of male copulatory behavior. The path followed by this chemosensory information has been relatively well documented and includes the cortico-medial amygdala and the bed nucleus of the stria terminalis [116,152]. As discussed above, *in vivo* tracing with CTB has revealed the presence in quail of an important projection from the arcopallium, and in particular the nucleus taeniae, to the POM [22]. Nucleus taeniae is the avian homologue of parts the mammalian medial amygdala [122,169,190], which suggests that olfactory inputs could reach the medial preoptic nucleus of the quail. The role of such information is, however, difficult to ascertain, based on the available experimental evidence presented above. If, as suggested by a few studies [37,92,107], olfactory information does play a role in the control of socio-sexual interactions, the projection from nucleus taeniae to POM would then acquire a particular importance in relation to this system.

These studies establish that the POM potentially receives both visual and olfactory information. It must be stressed, however, that the amount of sensory information that reaches the nucleus through these pathways has not been experimentally determined, and we do not know what type of cells in POM (e.g., steroid-sensitive or not) are connected to these inputs.

Complex outputs from the POM have also been identified. They reach the septum, the paraventricular (PVN) and the ventromedial nuclei of the hypothalamus, the tuberal region, the intercollicular nucleus (ICo), the PAG

and the VTA. Most of these structures have been implicated directly or indirectly in the control of copulatory behavior in mammals [116]. Many of these regions are also steroid-sensitive in quail and in mammals. They are presumably part of the neural circuitry that controls copulation. In particular, the connection with the PAG is potentially of utmost importance here. This area is massively connected in a bidirectional way with a large number of more caudal mesencephalic and pontine structures [29], and therefore, it appears to be an important center connecting anterior levels of the brain to brainstem nuclei involved in the control of the complex motor output represented in male copulatory behavior. The POM projection to PAG originates primarily from aromatase-immunoreactive cells, suggesting that these estrogen-producing neurons may play a direct role in the control of behavior [2].

6. Concluding remarks

Birds continue to be of interest to neuroendocrinologists investigating the hormonal and neural control of male sexual behavior. As reviewed in this paper, we have a reasonable grasp on the hormonal control of male sexual behavior in a few species, and we know many of the sites of action of steroid hormones relevant to these behaviors. The localization of the sites of T metabolism have also been described in detail in a few species. The central core of the circuit regulating avian male sexual behavior consisting of interconnections between the POA and the PAG is also reasonably well described. Future studies can usually go in two directions. One direction is to fill in gaps in the neural circuit. For example, how visual and auditory inputs can project to the POA and BST to regulate male behavior is largely unknown. Similarly, the projections from the PAG, which are essential for male sexual reflexes, are not well understood. Detailed tracing studies in rats, combined with identified neuroendocrine cell types and/or immediate early gene expression, have led to substantial progress in recent years [119,174]. Similar studies in birds can address questions of general interest that will help our thinking about the functional organization of the male sexual circuit. For example, will the PAG to nucleus paragigantocellularis projection that is so important for mammalian sexual responses [119] also be important in avian species that lack an intromittent organ? Will specialized visual and auditory projections to the POA be identified in avian species that are not present in mammals? Aromatase has been identified in sensory fibers in avian and mammalian species [81,102,121] and seems to be particularly present in brain areas directly connected to sensory inputs [78,103]. This observation raises intriguing questions about a direct hormonal regulation of sensory processing related to reproduction (see Ref. [24], this volume).

Second, comparative questions can also be addressed in birds in a way that would not be easy in other taxa. As we

learn more about the neuroendocrinology of avian male sexual behavior in a few species, precise hypotheses can be proposed about variation in this circuit that might be related to species variation in sexual behavior. In recent years, behavioral ecologists have been documenting a prodigious amount of variation in male copulatory behavior and courtship behavior that is viewed as an adaptation associated with the different mating strategies adopted by different species (e.g., Refs. [10,53]). The mechanistic basis of such variation is unknown and could be fruitfully studied to elucidate principles of brain-behavior evolution.

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