The Condor 91:966–978

BRAIN, HORMONE AND BEHAVIOR INTERACTIONS IN AVIAN REPRODUCTION: STATUS AND PROSPECTUS[1]

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Abstract. The central nervous system transduces behaviorally significant environmental information necessary for successful reproduction. Convergent lines of research suggest that a number of questions regarding the nature of species differences, and how these differences have been implemented over evolutionary time, can be approached through the clarification of underlying brain mechanisms. The last 15 years have yielded a host of techniques for studying the integration by the nervous system of sensory, neural, and endocrine factors mediating species typical reproductive behaviors. In this paper, we review major issues relevant to our present understanding of environmental-brain-endocrine behavior relationships in avian breeding systems, and indicate the techniques that are now available to explore neural mechanisms. Specifically, we discuss recent evidence on the localization of encephalic photoreceptors, the biological clock and its putative role in timing breeding cycles, peptidergic (gonadotropic hormone releasing hormone, GnRH, and vasoactive intestinal polypeptide, VIP) influences on the pituitary-gonadal axis, and the neurochemical consequences of hormone action in the avian brain.

INTRODUCTION

It has been known for centuries that stimuli from the environment profoundly influence reproductive physiology and behavior. Though many studies have documented the link between environmental changes and endocrine physiology, the mechanisms underlying these changes were not understood until well into the 20th century.

A major advance in our understanding of brain-hormone-behavior-environment interactions rested on the establishment of a route whereby the CNS could mediate the complex interaction between endocrine functioning and behavioral and environmental signals. In 1955, Geoffrey Harris demonstrated conclusively the existence of a humoral link between the brain and the pituitary gland via the pituitary portal system. This vascular connection is the route whereby signals from the brain reach the pituitary gland and thereby have access to the general blood supply of the body. We now know that such responses are mediated by the hypothalamo-hypophyseal-gonadal (HPG) axis. In particular, it now appears that these environmentally influenced reproductive phenomena are regulated by changes in hormones circulating in the blood (Marshall 1959, Lehrman 1961, Murton and Westwood 1977, Wingfield and Farner 1980, Follett 1984).

In the decades since Harris's discovery, we have learned a great deal about environmental factors regulating reproduction. A complete understanding of the ultimate causes of diverse reproductive strategies and of the evolution of associated mechanisms still eludes us. The classic issues of why and when animals do as they do, however, in terms of underlying hormonal mechanisms have been successfully addressed in both laboratory and field studies on behavioral, endocrine, and neural mechanisms of reproduction (Cheng 1979; Silver 1978; Silver and Cooper 1983; Wingfield and Farner 1980; Silverin 1983, 1988; Wingfield 1983; Wingfield et al. 1987).

Prominent features of the basic anatomical organization of the HPG are shared by all major vertebrate groups. Secretory neurons in the brain synthesize a hormone—gonadotropic hormone releasing hormone (GnRH)—which communicates with the hypophysis via a vascular connection—the pituitary portal system. Stimulatory glycoprotein hormones—gonadotropins—are secreted by the pituitary gland, which in turn initiate hormone secretion by the gonads. Gonadal steroids may stimulate or inhibit the functional status of the neural and/or pituitary components.

There is a distinct pattern of changes in

[1] Received 25 May 1989. Final acceptance 26 July 1989.

steroid and pituitary hormones correlated with behavioral and physiological events of the reproductive cycle (Silver 1978, Cheng 1979, Farner and Follett 1979, Wingfield and Farner 1980, Follett 1984, Wingfield et al. 1987). The temporal pattern of hormone secretion influences the progression from one stage of the reproductive cycle to the next. The onset of breeding is marked by an increase in the secretion of pituitary gonadotropins (luteinizing hormone—LH, and follicle-stimulating hormone—FSH) and gonadal steroids, androgens (such as testosterone and 5α-dihydrotestosterone) in males and estrogens (such as 17β-estradiol) in females. These hormones remain above nonbreeding baseline levels throughout the breeding season. In all species there is a rise in prolactin secretion associated with incubation and/or brooding (Goldsmith 1983).

Even though these hormone-behavior relationships have been established, the cause of the hormone changes and consequences of these endocrine changes remain to be fully explained. Also, knowledge of endocrine changes alone leave several other issues unresolved. Thus, surprisingly, significant differences in social behavior among species are associated with relatively small differences in hormone secretion. For example, two species with natural history patterns that vary from the typical avian pattern, the brood-parasitic Brown-headed Cowbird (*Molothrus ater*) and the polyandrous Spotted Sandpiper (*Actitis macularia*), have endocrine profiles of gonadotropins, sex steroids, and prolactin that are similar in many ways to those previously identified for monogamous biparental species. The Spotted Sandpipers exhibit reversed behavioral sex roles that are not based on a total reversal of the normal male to female ratio of androgens to estrogens (Fivizzani and Oring 1986). Brown-headed Cowbirds which remain sexually active throughout the breeding season show hormone profiles quite similar to parental species in the sexual phase of the annual cycle (see Dufty and Wingfield 1986 for review). In a similar vein, individual differences in behavior within a given population are generally not reflected by differences in hormone secretion in mammals (Damassa et al. 1977, Harding and Feder 1976), though at least one mammalian study has found such individual differences to be correlated with brain differences in hormone receptor levels (Clark et al. 1985). These issues related to the mechanisms underlying species differences and individual differences in reproductive cycles and reproductive behavior in birds may well be elucidated by examining the target sites for hormones in the brain.

NEURAL PROCESSES IN AVIAN REPRODUCTION

At the level of the brain one can study the integration of external environmental stimuli and internal bodily signals, and how these signals influence the neural substrate. A cascade of events are involved in this process: (1) specialized receptors for the external signals transduce the physical stimuli into neural events, (2) pathways from various receptors reach target areas in the brain, including the biological clock and neurons elaborating releasing hormones in the preoptic area and hypothalamus, (3) neuromodulatory and enzymatic factors regulate the releasing hormones, and (4) peripheral steroid hormones themselves act back on the brain to alter its responsiveness. To illustrate both the current status of our understanding, and the methods used to address these issues, we will discuss each of these events in turn.

RECEPTORS FOR ENVIRONMENTAL STIMULI

Temperate zone birds limit their reproductive activity to a particular time of the year. This involves responses to both photoperiodic information and to supplementary cues such as behavioral interactions, the availability of nest sites, nest material, and food (Wingfield 1983, Farner 1986). If we could identify the sensory receptors that transduce environmental cues this would represent an important first step in understanding how changes in hormone secretion are produced by the brain. This in turn would set the stage for understanding species differences in responsiveness to environmental factors, and the way in which mechanisms constrain the evolutionary lability of reproductive systems in birds. Of the relevant environmental stimuli, photic cues have been the most intensively studied.

Photoperiodic cues provided by daylength determine whether the reproductive system is in an active or inactive state, while supplementary cues accelerate or retard this process (Wingfield 1983, Follett 1984, Farner 1986). In birds, photoperiodic information is detected by an extraretinal encephalic photoreceptor while the supplementary information is processed by the ears and

eyes. Benoit (1935) demonstrated that blinded Mallard drakes (*Anas platyrhynchos* L.) continued to recrudesce their testes in response to artificial photostimulation. The existence of encephalic photoreceptors has been well documented in a variety of avian species (Oliver and Baylé 1980). One of the most recent analyses of the encephalic photoreceptor established an action spectrum for the photoperiodic response in Japanese Quail (*Coturnix japonica*) by presenting light at different wavelengths and measuring its effect on plasma luteinizing hormone (LH) levels. This work established that the photopigment mediating the LH response is very similar to rhodopsin (Foster and Follett 1985). Though the existence of these receptors had been known for decades, available neuroanatomical techniques were inadequate to determine the location and morphological features of these cells. For example, a search using electron microscopic methods for retina-like photoreceptors would in theory have been possible, but as this technique entails the analysis of almost microscopic blocks of tissue, it is unsuitable for surveys of large brain regions. A methodical search for brain photoreceptors became feasible with the application of the method of immunohistochemistry.

Immunohistochemical techniques have been applied to the brain since the late 1960s. These permit the localization of minute quantities of antigens, whether structural proteins or peptides and provide a new class of information about function and physiology. In this procedure, antibodies are used to localize a naturally occurring antigen present in appropriately fixed tissue. That is, antibodies are used as markers to label antigens naturally found in neurons. First, specific antibodies to the peptide or transmitter of interest are prepared. The antibody is then bound to the antigens in the tissue of interest. Next, a marker that can be visualized under the microscope is bound to the primary antibody. Finally the tissue is processed for light or electron microscopy. Methodological and interpretive aspects are reviewed in Sternberger (1979).

All known vertebrate photoreceptors contain the protein opsin as part of the photopigment that transduces the photic signal. Antibodies have been developed against opsin allowing the localization of this protein in the retina and other light sensitive structures such as the pineal. Using a monoclonal antibody that recognizes rod photoreceptors in a variety of vertebrates, Silver et al. (1988) described opsin-like immunoreactivity in cerebrospinal fluid-contacting neurons in the septal and tuberal hypothalamic regions of the dove and duck brain (see Fig. 1). These opsin positive perikarya also express another peptide, namely vasoactive intestinal polypeptide (VIP). Thus, for the first time, we have a potential anatomical marker for this brain photoreceptor. It should be noted that although this result is highly suggestive, it does not constitute definitive proof that these cells are the long sought encephalic photoreceptors. Ideally, an independent line of evidence indicating that the antigen stained is indeed opsin is desirable. For example, we are now using another anti-opsin antibody that recognizes a different epitope (antigenic site) on the opsin molecule. Another important step is to establish that the opsin-bearing cells can respond to light.

The use of anti-opsin antibodies can guide us to circumscribed neuroanatomical locations of encephalic photoreceptors. Once the location of photoreceptor(s) is (are) established further functional investigations can indicate how the signal produced by this photoreceptor in response to light is conveyed to GnRH neurons and to the biological clock that measures daylength. Subsequently, one can determine whether species differences in response to photoperiod are reflected in the anatomy and/or physiology of the photoreceptor and its efferent connections. It will be fascinating to compare the neural circuitry mediating the environmental response of the reproductive system in temperate zone photoperiodic species with species that breed opportunistically in response to other cues such as rainfall.

THE BIOLOGICAL CLOCK IN BIRDS

Changes in daylength, constituting one of the primary environmental factors regulating seasonal cycles of gonadal growth and regression, appear to be measured in birds by means of a biological clock. As illustrated first by Hamner (1963), birds seem to use the phase of an endogenous circadian oscillator rather than the absolute duration of light to measure photoperiod. In experiments of this design the amount of light is held constant, while the length of the dark period is varied among the experimental groups. The results indicate that there is a circadian rhythm in sensitivity to light, such that birds receiving light in phase with the hypothesized 24-hr cycle in photosensitivity show gonadal growth while birds receiving an equally long light signal out of synchrony with this sensitive phase

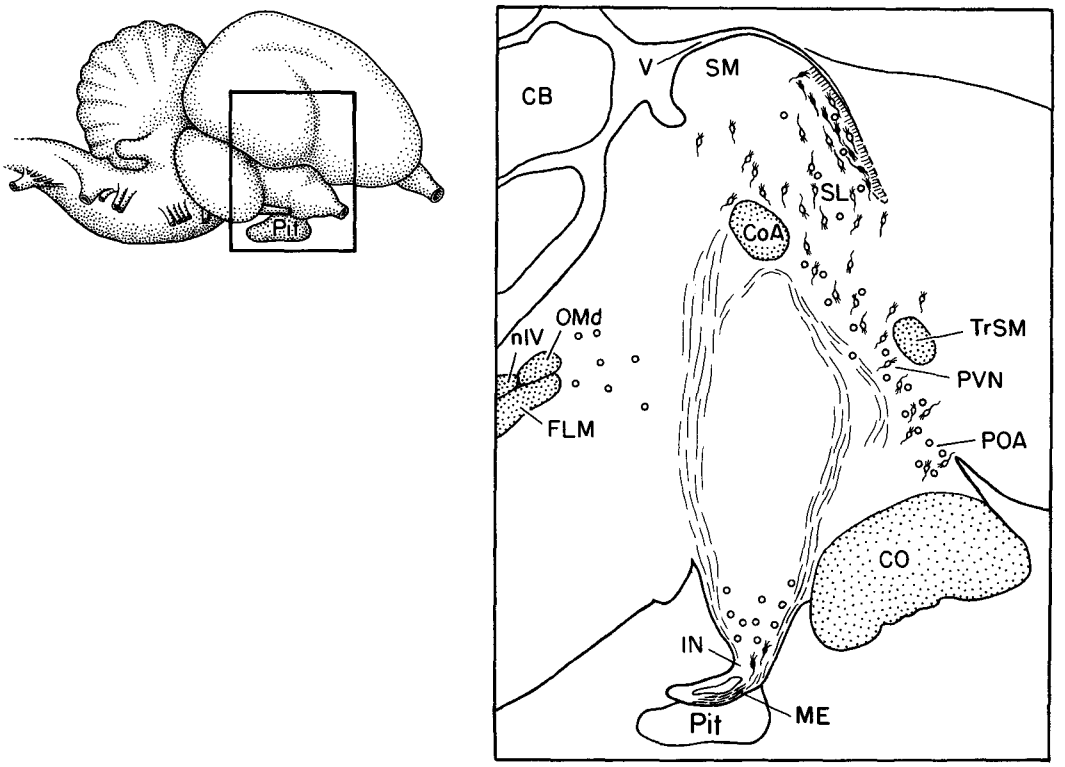

FIGURE 1. A drawing of a "generic" avian brain illustrating some of the neurochemical systems discussed in the text. The box outlining a subset of the three-dimensional schematic brain on the left denotes the area enlarged and shown in detail on the right. This schematic presents the hypothalamic and infundibular area of a "generic" avian brain cut in the sagittal plane. The dark neuron-like cells illustrate opsin-like immunoreactive cells in the lateral septum (SL) and infundibular region (IN). Note the dark lines running along the lateral ventricle in the lateral septal region. This denotes the fact that the cells containing opsin-like immunoreactivity are located in the lateral ventricular wall of the lateral septum. These cells are candidates for the cells that may contain the extra-retinal photoreceptor. These data are based on information contained in Silver et al. 1988. The open neuron-like cells that are scattered rostrally from the POA and run in a column caudo-dorsally until the medial septum illustrate the localization of immunoreactive perikarya containing the pituitary regulating peptide gonadotropin-releasing hormone (GnRH). The dashed lines running ventrally in two columns represent the major fiber pathways that contain this peptide and innervate the median eminence and ultimately are released into the portal system and regulate pituitary functioning. This distribution pattern is based primarily on information contained in Foster et al. 1987. The open circles that are scattered throughout the POA, PVN, SL, as well as ventrally in the IN and caudally near the OMd represent the distribution of neurons containing estradiol receptors. This information is based primarily on data contained in Martinez-Vargas et al. 1976. Abbreviations listed in the figure are as follows: CB: Cerebellum; CoA: Commissura anterior; CO: Chiasma opticum; FLM: Fasciculus longitudinalis medialis; IN: Nucleus infundibuli hypothalami; ME: Eminentia mediana; nIV: Nucleus nervi trochlearis; OMd: Nucleus nervi oculomotorii, pars dorsalis; Pit: pituitary gland; POA: Nucleus preopticus anterioris; PVN: Nucleus paraventricularis magnocellularis; SL: Nucleus septalis lateralis; SM: Nucleus septalis medialis; TrSM: Tractus septomesencephalicus; V: Ventriculus lateralis.

show no gonadal development (Hamner 1963, Follett et al. 1974, Turek 1974, Tewary and Kumar 1981). The results suggest that a biological clock or oscillator is necessary for the measurement of daylength (however, see Saiovici et al. 1987 for an alternative view). Little is known about the physiological basis of such an entity.

The pineal gland, the eyes, and the hypothalamic suprachiasmatic nucleus (SCN) have all been suggested as sites for the biological clock (Cassone and Menaker 1985). However, in contrast to the situation in mammals, most data suggest a minor role for the pineal gland in the mediation of photoperiodic effects in birds (Follett 1984, Gwinner 1986). Also, as noted previously, it has been shown in several avian species

that the eyes are not necessary for the exhibition of photoperiodic responses (Follett 1984, Underwood 1975). In mammals the existence of a biological clock in a hypothalamic SCN is supported by several independent lines of evidence (Rusak and Zuker 1979; Moore 1982, 1983; Lehman et al. 1987). These consist of: (1) lesions of the SCN abolish circadian rhythms in physiological and behavioral activity and result in the loss of photoperiodic responses to daylength, (2) direct retinal input to the SCN mediates the entrainment (synchronization) of reproductive and daily activity cycles produced by light cues, (3) circadian cycles of multi-unit electrical activity within the SCN both in vivo and in vitro, (4) daily cycles of glucose metabolic activity in the SCN in the absence of photic cues, and (5) restoration of circadian rhythmicity by SCN transplants of fetal donor tissue to an arrhythmic animal.

Initial studies of the avian circadian system were directed at a medial hypothalamic nucleus lying in the same region as the mammalian SCN (overlying the optic chiasm and adjacent to the third ventricle). The results in Japanese Quail (Simpson and Follett 1981), House Sparrows (*Passer domesticus*) (Takahashi and Menaker 1982), and Java Sparrows (*Padda oryzivora*) (Ebihara and Kawamura 1981) suggested that lesions of this nucleus resulted in the loss of locomotor rhythmicity but did not disrupt photoperiodic responses (Simpson and Follett 1981). However, at least in the case of House Sparrows, hypothalamic lesions were large and resulted in a decrease in overall activity level (Takahashi 1981), raising the possibility that the effects were not specific.

One feature of the mammalian SCN is direct input from the retina (Moore 1973). A clue to the location of the SCN in birds might be to identify the retino-recipient nucleus. Older methods for such an identification involved the tracing of degenerating fibers following the transection of nerves of tracts. While this technique was adequate for large fiber bundles, degeneration of very fine fiber bundles is harder to identify. The development of sensitive tracers such as horseradish peroxidase (HRP) has allowed the resolution of fine neural connections.

HRP is an enzyme taken up by neurons and transported (200–300 mm/day) in the retrograde and anterograde direction (Mesulum 1983). Appropriately fixed tissue is visualized using the chromogen diaminobenzidine (DAB) or tetramethylbenzidine (TMB). When hydrogen peroxide is added to the medium containing floating tissue sections, the liberated oxygen combines with the benzidine to make a pigment which can easily be visualized under the microscope. (Other sensitive tracers are available, see Nauta and Feirtag 1986 for discussion.)

Application of HRP tract-tracing techniques in a number of avian species has identified a retino-recipient region in the lateral hypothalamus in many avian species (Norgren and Silver, in press). Only one behavioral study has been directed specifically to this nucleus. Lesions of the lateral hypothalamic retino-recipient nucleus do not affect circadian rhythmicity in pigeons (Ebihara et al. 1987). It is possible that the large hypothalamic lesions of Takahashi and Menaker (1982) in House Sparrows included this region (Takahashi, pers. comm. with RS). The absence of the several lines of evidence necessary to establish the existence and location of a neural oscillator in the hypothalamus of birds (as are available in mammals) represents a significant hiatus in our knowledge.

HYPOTHALAMIC RELEASING FACTORS

Once environmental stimuli are transduced by the appropriate receptor, they influence the secretion of the peptide GnRH located in neurons of the preoptic and hypothalamic regions (see Fig. 1 for the distribution of these cells). The avian brain contains two forms of this peptide (Millar and King 1984, Miyamoto et al. 1984, Sherwood et al. 1988) known as avian GnRH I and II; however, all the available evidence suggests that only GnRH I is involved in the control of pituitary hormone secretion (Sharp et al. 1988). GnRH regulates the activity of the two pituitary gonadotropins, LH and follicle-stimulating hormone (FSH).

Studies of GnRH positive neurons by radioimmunoassay (Dawson et al. 1985) and by immunohistochemistry (Foster et al. 1987) reveal striking differences in the activity of this peptide in reproductively active vs. inactive European Starlings (*Sturnus vulgaris*). Radioimmunoassay allows the determination of the amount of a hormone present in a given piece of tissue or volume of plasma. From these data it appears that the GnRH content of the POA-hypothalamus is 10-fold higher in photosensitive (i.e., capable of responding to long daylengths) compared to pho-

torefractory (i.e., incapable of responding to long days) birds.

Immunohistochemical evidence indicates the location of the GnRH cell bodies in the preoptic and septal regions of the brain and confirms a seasonal change in the intensity of staining. Individual neuronal perikarya in the preoptic area increase in diameter and in the intensity of GnRH staining density in photosensitive animals. Thus seasonal cycles of sensitivity to daylength are accompanied by changes in the synthesis of GnRH. The induction of gonadal growth associated with photosensitivity (or the breaking of photorefractoriness) is preceded by an increase in hypothalamic GnRH content (Goldsmith et al. 1989). Similarly, gonadal regression is preceded by a decline in GnRH (Goldsmith et al. 1989). These results indicate that the factors regulating this neuropeptide are the key to understanding the control of seasonal processes. Additional studies employing tract tracing to determine how environmental information reaches the GnRH neurons, in combination with chemical neuroanatomical methods, will provide information on neuroendocrine integration.

VIP is another releasing factor that has recently been implicated in the control of a pituitary hormone, prolactin. This hormone is modulated by stimuli from the environment and plays an important role in the control of reproduction. VIP stimulates the secretion of prolactin by the anterior pituitary (Macnamee et al. 1986). Exogenously administered VIP releases prolactin when administered to intact birds (Lea and Vowles 1986). In vitro studies also point to VIP as directly stimulating the release of prolactin from the anterior pituitary (Macnamee et al. 1986). Incubation is associated with elevated plasma prolactin in birds (Goldsmith 1983, Lea 1987). In order to establish whether VIP mediates normally occurring changes in prolactin secretion it is necessary to demonstrate that changes in VIP precede changes in prolactin. As noted earlier, VIP also is co-expressed in cells that contain opsin-like immunoreactivity. However, VIP is widely distributed throughout the hypothalamus of birds and we do not at present know if these VIP-positive cells are involved in the regulation of prolactin.

Differences between incubating and nonincubating birds in the intensity of VIP staining in the hypothalamus have been described in pigeons, turkeys, and doves (Peczeley and Kiss 1988, Mauro et al. 1988). In Ringed Turtle-Doves (*Streptopelia risoria*), Cloues et al. (1988) measured changes in VIP cell diameter throughout the reproductive cycle. The results indicate that VIP cell size starts to increase during courtship, peaks around the ninth day of incubation, remains elevated until day 14 of squab rearing, and then slowly declines as the squabs become independent. Elevation of plasma prolactin in the Ringed Turtle-Dove are detected only at mid-incubation (Goldsmith et al. 1981, Cheng and Burke 1983) indicating that VIP immunoreactivity precedes these plasma hormone changes. Furthermore, parent doves rearing two squabs have a more prolonged period of enlarged VIP immunoreactive cells compared to parents rearing a single squab. This parallels differences in crop development between these two groups. Similarly, inexperienced birds, who feed their young and secrete prolactin for longer periods of time than experienced birds, have a prolonged period of enlarged VIP immunoreactive cells compared to parents with previous experience of a reproductive cycle, again paralleling differences in crop growth between these two groups. It has long been known that inexperienced birds are less efficient at rearing their young than are experienced birds. To our knowledge, this is the first demonstration that such experiential effects are reflected in neuroendocrine cells. Control animals paired with the same sex partners or without access to nest bowl and straw showed no changes in VIP immunoreactivity.

The timing of prolactin secretion varies among species and even within species as a function of the pattern of parental care (Goldsmith 1983). It will be useful to assess whether species differences and sex differences in temporal aspects of prolactin secretion are accompanied by differences in the activity of VIP. If this proves to be the case, studies of VIP immunoreactivity could direct us to higher neural processes underlying species differences in behavior.

As discussed in the introductory paragraphs, the brain integrates sensory and endocrine information to regulate the secretion of pituitary and gonadal hormones. In this section we have concentrated on three neural components of reproductive processes in birds: the encephalic photoreceptor, the biological clock, and neural peptidergic and protein hormones. Much work on the anatomy and physiology of each component remains to be done. Also, connections

among these neural entities are very poorly understood. In the next section, we will review the ways in which steroid hormones act on the neural substrate.

We will concentrate on the sex steroid hormones, testosterone, 17β-estradiol and progesterone, as they have been the most extensively studied. However, it should be noted that progress is being made in understanding where and how other hormones such as the pituitary hormone prolactin act in the brain (Buntin and Walsh 1988). We will further limit the discussion to steroid hormone influences on the adult brain. But, it should be recognized that the developmental action of steroids in organizing neural circuits is an important factor underlying interspecific differences in brain and behavior (see Arnold et al. 1986 for discussion).

NEURAL SITES OF STEROID ACTION

Steroid hormones exert their effects in discrete areas of the brain where specialized receptors are located. The development of autoradiographic methods in the late 1960s allowed for the identification of these hormone sensitive sites (see Fig. 1).

Autoradiographic methods were first widely applied to steroid hormones, such as the gonadal sex steroids. Autoradiography requires the removal of endogenous sources of the steroid (usually by gonadectomy) followed by the injection of the radioactively labeled hormone of interest. The brain tissue is then placed on emulsion coated slides. After exposure to the film (4 to 12 months) and development (similar to photographic development) one can visualize the location in the brain of the radiolabeled hormones (Morrell and Pfaff 1981).

In birds some of the first studies of this sort were conducted by Martinez-Vargas et al. (1974, 1975, 1976) in their investigations of estrogen receptor binding in Ringed Turtle-Doves. The method was subsequently applied to a number of other species representing several orders (Arnold et al. 1976, Zigmond et al. 1980, Watson and Adkins-Regan 1989). The results indicate that in hypothalamic and preoptic areas the pattern of steroid receptor binding in birds corresponds to the general vertebrate pattern (Morrell and Pfaff 1981). The one exception to this rule is in the uniquely avian, steroid sensitive, sexually dimorphic complex of nuclei involved in the motor control of song that appears to be specific to members of the oscine suborder (Arnold et al. 1976). However, consistent with the vertebrate pattern is the presence of steroid receptors in the septum, archistriatum, preoptic region, tuberal hypothalamus, specific subtectal loci (e.g., the nucleus intercollicularis), and pituitary gland. Many of these regions contain both androgen and estrogen receptors. More recently, antibodies to steroid receptor proteins have been developed permitting the use of immunohistochemical techniques (Gahr et al. 1987, Ball et al. 1981a, Balthazart et al. 1989). To date, these studies have been in agreement with results stemming from the autoradiographic work. Immunohistochemical techniques provide the advantage of identification of cellular components (nucleus vs. cytoplasm) in which steroid receptors are located. Immunohistochemical techniques also allow double labeling of cells for the presence of both steroid receptors and neuropeptides or transmitters, permitting analysis of how these chemical messengers interact.

STEROID-INDUCED NEUROCHEMICAL CHANGES

The identification of steroid-concentrating brain regions guides us to the analysis of how and where steroids act. Lesion and steroid implant studies to steroid-concentrating brain regions indicate that these areas are necessary for the performance of reproductive behaviors such as copulation, certain courtship displays, and song (Hutchison 1978, Nottebohm 1980). With respect to parental care, little work on its neural basis has been done in birds.

One way that sex steroids affect behavior is by modifying neurotransmission in steroid-concentrating brain regions (Dohanich et al. 1985, McEwen et al. 1987). The actions of steroids have been best characterized for the case of estradiol acting in the ventromedial nucleus of the rat (Pfaff 1980). Though the actions of steroids on neurochemical functioning have been much better documented in mammals than in birds, many of the major ways in which steroids modify neurotransmission have also been demonstrated in birds in studies of copulatory and vocal behavior. For example, steroids profoundly affect the activity of the monoamine neurotransmitters, as measured by steroid-induced changes in transmitter "turnover."

Turnover refers to the overall rate at which neurotransmitters or neuropeptides stored in a given tissue are replaced. It can thus provide an index to the func-

tioning of catecholamine neurotransmitters such as norepinephrine, epinephrine, and dopamine. Turnover for a catecholamine such as norepinephrine can be detected by measuring the disappearance rate of endogenous norepinephrine after the inhibition of catecholamine synthesis by an inhibitor of the rate limiting enzyme tyrosine hydroxylase (see Cooper et al. 1986 for discussion of this method). The transmitter level itself can be measured by a variety of methods. One of the most sensitive approaches allows for the detection of transmitter levels in microdissected "punches" of tissue from individual brain nuclei using sensitive high performance liquid chromatography with electrochemical detection methods (see Renner and Luine 1984 for a detailed description).

A modification in turnover can be achieved by a variety of different mechanisms. One such pathway is through the enzymes ultimately responsible for the amount of transmitter available to the neuron. Steroids can also influence the receptors available for the neurotransmitter interaction. In birds relatively few studies have examined changes in neurotransmitter turnover, either in response to a hormone cue or in response to an environmental stimulus such as light. Ottinger and Balthazart (1987) as part of a series of studies designed to establish how testosterone induces copulatory behavior in the male Japanese Quail have found that the removal of endogenous testosterone by castration leads to a decrease in the turnover rate of norepinephrine that is restored to intact levels by testosterone in males while opposite effects are observed in females. Similarly, steroids have been shown to induce changes in neurotransmitter functioning in the song system of Zebra Finches, *Poephila guttata* (Barclay and Harding 1988a). Steroids were found to induce different changes in catecholamine turnover in the various vocal control and hypothalamic nuclei (Barclay and Harding 1988b).

Steroids are known to modify enzymes associated with the regulation of neurotransmitter functioning in birds (Luine et al. 1980). For example, in Zebra Finches castration results in a decrease in the activity of two cholinergic enzymes in peripheral nerves that innervate the syrinx (Luine et al. 1980). Replacement with testosterone reinstates the activity of one of these enzymes, suggesting that testosterone may regulate singing behavior in passerine birds through the induction of enzymatic proteins in testosterone target neurons and muscles.

Another component in the neurochemical communication process that can be modified by steroids to change neurotransmitter activity and ultimately behavior is the availability of receptors for a particular transmitter. The density of neurotransmitter receptors has been found to be different between the sexes (Ball et al. 1989b) and to be modulated by steroids (Balthazart and Ball 1989) in certain discrete brain regions as determined by the method of quantitative autoradiography.

Quantitative autoradiography for neurotransmitter receptors is broadly similar to the autoradiographic procedures described above for steroid hormone uptake. However, in this case in vitro labelling procedures are employed and a computer assisted image analysis is used to analyze the isotope sensitive film allowing for the quantification of the density of receptors in a given brain area as well as for a description of their localization (see Kuhar 1985 for description of method).

In the Japanese Quail it has recently been demonstrated that in certain steroid-concentrating brain nuclei there is a sex difference in the density of the alpha$_2$ receptor subtype for the neurotransmitters norepinephrine and epinephrine (Ball et al. 1989b). For example, the density of receptors is higher in males than in females in the dorsal portion of the infundibulum, an area implicated in the regulation of LH and in the midbrain nucleus intercollicularis, an area important in the control of vocal behavior (Ball et al. 1989b). In the dorsal infundibulum, castration leads to an increase in the density of alpha$_2$ adrenergic receptors and testosterone reverses this effect (Balthazart and Ball 1989). The sex difference in receptor binding in this region and the regulation of these receptors by testosterone parallel the situation for pituitary hormone LH, in that there are higher circulating plasma levels of LH in males than in females (Urbanski and Follett 1982) and levels increase after castration and are reduced by testosterone. These findings suggest that alpha$_2$ in the dorsal infundibulum could play a role in the steroid feedback control of LH.

Steroid modification of neurochemical activity appears to be limited to discrete areas of the brain. An advantage of analysis of the avian brain is that a number of steroid-modified neurochemical systems are found in nuclei that have well defined behavioral functions. It should be noted that the most detailed information on how a steroid action in the brain results in a behavioral response is only available for estrogenic effects in the ventromedial nucleus of the rat (Pfaff 1980).

In all the avian systems described above, the cascade of events from steroid binding to neurotransmitter and enzyme systems and the behavior remain to be fully described.

Analysis of the steps in developing the chain(s) of causal events among steroid hormone action, neurochemical functioning, and behavior has begun in birds. Several avian reproductive behaviors such as copulatory behavior and song have well characterized neural loci in birds. There is a wealth of species differences among birds. The analysis of avian neural mechanisms mediating reproduction is a potentially valuable source of comparative data on how variations in hormone action relate to variations in behavior.

THE NEURAL ENVIRONMENT MODIFIES HORMONE EFFECTIVENESS

The manner in which a hormone such as testosterone modifies neural function and behavior is profoundly influenced by the nature of the substrate on which it acts. Before steroid hormones exert their effects they may be metabolized to active or inactive forms. Thus the density of metabolizing enzymes in a specific tissue at a given time will greatly determine whether or not a hormone has any effect at a particular target site. The interaction of the brain enzyme environment with steroid hormone effectiveness has been characterized more effectively in the avian brain than in any other vertebrate system (Hutchison and Steimer 1983).

The technique for measuring changes in enzyme activity utilize indirect radioenzymatic assays (Schumacher and Balthazart 1987). Here tissue is sectioned (300 μm) and the area of interest is microdissected using a method called "punch." Tissue is then homogenized and incubated with radioactive testosterone. The radioactive steroids are then extracted and purified. By counting the amount of estradiol generated from the testosterone by the substrate, one can infer the amount of aromatase (an enzyme that converts testosterone to estradiol) present in the tissue. While useful for quantification, these microdissections provide only a crude index of the location of the enzyme. Development of antibodies (in the future) to the enzyme will allow more precise (immunohistochemical) localization of aromatase enzyme.

In quail, male copulatory behavior is androgen dependent. It disappears completely after castration and is restored after androgen treatment (Adkins and Adler 1972, Schumacher and Balthazart 1983). This effect of testosterone cannot be induced in females even after administration of pharmacological doses of testosterone. Interestingly, circulating levels of androgens are only slightly lower in female than in male quail, with an occasional overlap in male and female values (Doi et al. 1980, Balthazart et al. 1986). The activation of male sexual behavior by testosterone requires that testosterone be metabolized to estradiol by the enzyme aromatase. It appears that this behavioral dimorphism in quail depends on differential properties of brain areas involved in the control of behavior. In particular aromatase is more active in males than in females throughout the hypothalamus and especially in the preoptic area (Schumacher and Balthazart 1986). The enzyme 5β-reductase, which is more active in females, metabolizes testosterone into a biologically inactive form of the hormone, 5β-dihydrotestosterone.

The evidence described above indicates that sex differences in the ability of testosterone to induce copulatory behavior rests on a sex difference in the steroid metabolizing ability in the brain. An obvious question to pursue is whether species differences in the effectiveness of steroids in inducing reproductive responses might also lie in brain enzyme differences. Thus in the Spotted Sandpiper, it may be that the reversal of sex roles in behavior (which is not accompanied by sex differences in hormone levels) may instead be accompanied by sex differences in metabolizing enzymes.

SUMMARY

In this review, we have tried to highlight some of the major recent advances in our understanding of the brain mechanisms regulating the reproductive cycle in birds. While it is true that in some areas of research it is the study of mammals which has led to the development of new techniques and principles, it is often true that avian forms provide the most dramatic examples of environment-brain-hormone-behavior integration. This derives in part from the availability of information on environmental ecology of many different avian species, and on diverse behavioral adaptations shown by birds. In birds, we can study the neural and endocrine basis of brood parasitism, monogamy, polygyny, polyandry, diverse breeding cycles, and their regulation by a variety of environmental cues.

Understanding how the brain mediates the response of the endocrine system to the constantly changing physical and social world is a major problem now facing neuroendocrinology and reproductive biology. We anticipate that the wealth of information available on specific avian adaptations will provide insight into general principles of neuroendocrine processes.

ACKNOWLEDGMENTS

We thank M. Morton and his students for delaying their Thanksgiving dinner to make comments on previous drafts of this paper. We also thank Michael Numan for reading and commenting on the paper. The research on Ringed Turtle-Doves from the laboratory of RS was supported by NIMH grant 29380. The figure was drawn by L. Laszlo Meszoly.

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