Evolution of Brain Mechanisms Controlling Sexual Behavior

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I. Introduction

The comparative perspective (i) offers insight into the rules, principles, or
generalizations that govern the structure and function of the brain and (ii)
illuminates the roots from which they emerged. For example, it is reason-
able to assume that traits or characteristics shared by many different verte-
brates are evolutionarily more ancient, and hence more fundamental, than
those traits less widely shared and hence less fundamental. This is known as
the biogenetic law and is central to the theoretical insights of the great neu-
rologist J. Hughlings Jackson. Information on what is common (fundamen-
tal) and what is uncommon (recently derived) can best be gathered by
studying a variety of species, including nonmammalian species. Further, atypical organisms can be especially useful, for their unusual adaptations illustrate alternative solutions to particular problems. Also, they "often force one to abandon standard methods and standard points of view" with the result that "in trying to comprehend their special and often unusual adaptation, one often serendipitously stumbles on new insights." Finally, studying reptiles, the present-day representatives of the ancestors of mammals and birds, yields insight into the origin and adaptation of neuroendocrine mechanisms of species-typical behavior in the higher vertebrates.

The primary question addressed in my (D.C.) work concerns how brain mechanisms controlling behavior might evolve. That is, how has the brain come to exploit specific external and internal stimuli so that they serve as triggers for adaptive responses? A basic tenet of neuroethology is that the structures and functions of the central nervous system are adaptational responses to the environment. While we know that the neural mechanisms underlying behavior can be modified by mutation, evolutionary selection pressures, or by hybridization of closely-related species, we know very little about how brain-behavior relationships evolved. This is of considerable interest and fundamental importance to our understanding of the neural control of complex behaviors, but it is difficult to address for three reasons:

1. To demonstrate that microevolutionary changes in the neural mechanisms controlling a specific behavior are a result of selection, it is necessary to establish both that individuals inherently differ in their performance of the behavior, and that these differences in behavior lead to differential reproductive success. Only when these facts have been established can the issue of differences in mechanism be meaningfully addressed.

2. We rarely know the exact phylogenetic relations among the species at hand. Even though closely-related species may be compared, the common ancestor to these species usually no longer exists, and further, there is no way of determining the exact number of intervening species since the original divergence.

3. Behavior-genetic analyses such as screening for mutants or selective breeding reveal the potential for the brain to change in response to artificial selection, not how the brain responds to the selection pressures present in nature.

The end result is that we often are only able to make assumptions regarding the patterns of evolutionary change.
II. Initial Studies (1978–1987)

In 1978, David Crews and Kevin Fitzgerald independently observed two lizards mating. Ordinarily this would not be a notable event. It was serendipitous however because we knew that both animals were females and, further, that the species consisted entirely of females, reproducing by obligate parthenogenesis. What was remarkable was that the behaviors exhibited by the parthenogenetic whiptail were identical to the courtship and copulatory behavior of its direct sexual ancestor. On seeing the animals engaged in this behavior, since termed pseudosexual behavior, it struck one of us (D.C.) that this display of both male- and female-like “sexual” behaviors alternately by a single individual demonstrates perfectly the fundamental bisexuality of the vertebrate brain. Simultaneously hermaphroditic species make the same point in that individuals alternate in their behavioral roles, although in such species the gonad is an ovotestis that releases both sperm and eggs.

Since that time the Crews Lab has been studying whiptail lizards (genus Cnemidophorus) because they afford a particularly good opportunity to investigate the evolution of the neuroendocrine mechanisms underlying reproduction. (In this section references to individual facts are not cited; rather we refer the reader to a review\(^a\) for specific citations). This is because a direct ancestor-descendant phylogeny is present and two different forms of reproduction exist, sexual and asexual. Approximately one-third of extant whiptail lizard species are all-female (parthenogenetic) species that resulted from hybrid unions of sexual species. For example, the parthenogenetic desert-grasslands whiptail \((Cnemidophorus uniparens)\) descended from a hybridization event between two sexually reproductive species, the rusty rumped whiptail \((C. burtii)\) and the little striped whiptail \((C. inornatus)\); two-thirds of the triploid genome of the descendant parthenogenetic species is derived from the little striped whiptail, the maternal ancestral species.

Although genetically similar, the desert-grasslands whiptail (hereafter the parthenogenetic or descendant whiptail) and the little striped whiptail (hereafter the sexual ancestor whiptail) differ in several aspects of their reproductive biology. For example, (i) estradiol \((E_2)\) concentrations in reproductively active parthenogenetic whiptails are approximately five-fold lower than in reproductively active female sexual whiptails, and (ii) while the sexual ancestral species display the typical vertebrate pattern in that the male mounts and intromits and the female is receptive to the male’s courtship and copulation, individual parthenogenetic whiptails alternate between displaying male-like pseudocopulatory behaviors and female-like receptive behaviors depending upon the stage of follicular development (Figure 8.1).
Our early experiments indicated that engaging in pseudosexual behavior stimulates ovarian growth in the parthenogenetic whiptail Cnemidophorus uniparens just as male courtship stimulates ovarian growth in its sexual ancestor, C. inornatus. In both species, the time to the first ovulation is decreased significantly if sexual (or pseudosexual) behavior is present. Indeed, females of the sexual species will only lay eggs if a sexually active male is present; females housed with a male that fails to court because he has been castrated neither ovulate nor lay eggs. Similarly, in the parthenogenetic whiptail, engaging in pseudosexual behavior increases the likelihood of ovulation. Over the course of a reproductive season this effect can be substantial. Isolated parthenogens will eventually ovulate, but it is rare that they will
produce no more than one clutch. Two intact parthenogens housed together will each produce usually two or more clutches. If a parthenogen is caged with another parthenogen who has been ovariectomized and hormonally treated so as to only exhibit male-like behavior, the intact individual will produce two and sometimes three clutches (this is the same number of clutches produced in nature). Thus, study of sexual and unisexual whiptail lizards meets two major challenges in evolutionary studies — namely, demonstration of differential reproductive success and presence of the ancestral species.

Pseudo sexual behavior in parthenogenetic whiptails is related to the ovarian cycle (Figure 8.1). Female-like receptive behavior is limited to the preovulatory stage of the follicular cycle, whereas the expression of male-like mounting behavior occurs most frequently during the postovulatory stages of the cycle. Differences in the behavioral roles during pseudocopulations are paralleled by differences in the circulating levels of sex steroid hormones. That is, individuals show primarily female-like behavior during the preovulatory stage when E_2 concentrations are relatively high and progesterone (P) levels are relatively low; in contrast, individuals display male-like behavior in the postovulatory phase when concentrations of E_2 are low and P levels are high.

In the sexual ancestral species, sexual activity in males is dependent on testicular androgen, and administration of exogenous androgen stimulates male-like pseudo sexual behavior in the unisexual whiptail. This led to the expectation that androgen levels would be elevated during the male-like phase of the ovarian cycle. However, it was a surprise to find that androgens remained uniformly undetectable in the parthenogen throughout the reproductive cycle. In addition, the nature and pattern of sex steroid hormone secretions in the parthenogens are virtually identical to the females of the sexual species. Together these data indicate that the evolution of parthenogenesis and pseudo sexual behavior have not been accompanied by an alteration of the female-typical pattern of endocrine changes.

Changes in behavior commonly occur at transitions in circulating levels of hormones. The close parallel between the transition from female- to male-like pseudo sexual behavior and from E_2 dominance to P dominance in the circulation at ovulation (Figure 8.1) suggested that this shift in hormone concentrations may play a crucial role in controlling the expression of male-like pseudo sexual behavior. To test this hypothesis, parthenogens had their ovaries removed and were then given a P or E_2 implant or a blank capsule. Animals were paired with another similarly ovariectomized parthenogen that had, or had not, received hormone treatment. The results were clear-cut. Pseudocopulations occurred only in pairs in which both individuals were hormone-treated in a complementary fashion (e.g., in pairings of E_2- and P-treated individuals). Further, in all pseudocopulations, the P-treated parthenogen assumed the male-like role while animals treated with E_2 exhibited the female-like role. In the absence of the appropriate hormones, pseudo sexual behavior was never exhibited. Thus, it appears that the postovulatory surge in P has been exploited as the hormonal cue triggering male-like pseudo sexual behaviors.

During the last decade the focus of the laboratory has been on individual variation in the capacity of P to induce male-typical behavior (= P-sensitivity), the evolution of underlying mechanisms, and structure-function relationships in brain and behavior. (A) It was first documented that there exists individual variation in sensitivity to P in males of the sexual ancestral species. This work suggests that individual variation in P-sensitivity in the sexual ancestor served as the substrate for the evolution of P-activation of male-like pseudosexual behavior in the descendant unisexual species. Work with mammalian models (rats and mice) revealed that this role of P in modulating sexual behavior of males may be widespread. (B) To study the evolution of the molecular neuroendocrine events regulating sexual and pseudosexual behavior, the neuroanatomical distribution of estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) mRNA, the sensitivity to circulating hormones, and the regulation of ER and PR mRNA by hormones in the sexual and unisexual species were characterized. (C) Finally, the potential for structure-function relationships between sexual dimorphisms in behavior and sexual dimorphisms in the hypothalamus was investigated. Detailed reviews of these findings along with specific citations can be found in Godwin and Crews and Young and Crews.

A. Individual Variation in Progesterone Sensitivity

Given the cost that the evolutionary loss of a central nervous system structure would seem to entail, it is easier to simply evolve another system of controls. That is, the structure remains, but the agent activating that structure changes. In terms of the male-like pseudosexual behavior in parthenogens, this would mean that a female physiology must become capable of stimulating specific brain areas to express male-like behaviors at the appropriate time. For this to happen, there must exist a predisposition for such novel functional relationships in the ancestral species.

Earlier experiments established that mating behavior in males of the ancestral sexual species is dependent upon testicular androgens (see Reference 6). How could an androgen-dependent male-typical behavior of the sexual ancestral species evolve to become a P-dependent male-like behavior in the unisexual descendant species? Existing features can be produced by two distinct historical processes. One is adaptation, or the gradual selection of traits resulting in improved functions. Some traits, however, evolved from features that served other roles, or had no function at all, and were co-opted for their current role because they enhance fitness. This latter process may be termed exaptation. In adaptation, traits are constructed by selection for their present functions, while exaptations are co-opted for a new use.
In the present case, variation in P-sensitivity among male whiptails of the ancestral sexual species appears to be the substrate on which selection operated, resulting in the novel hormone-brain-behavior relationship observed in the parthenogen. That is, it was found that in approximately one-third of the males of the sexual ancestral species exogenous P was capable of stimulating or maintaining sexual behavior; further, the majority of males that were vigorous courters were P-sensitive, whereas none of the low courting males were P-sensitive (see Reference 6). Assuming that a P-sensitive male was involved in the hybridization process, the postovulatory surge in P presents a reliable stimulus that, given the low circulating concentrations of androgens, was co-opted to trigger mounting behavior in the parthenogen.

Named for its central role in female reproduction, P traditionally has been thought to have little or no function in the control of sexual behavior in males. Indeed, early experiments indicated that administration of P to male birds and rats will inhibit their sexual behavior.\textsuperscript{11-14} In fact, this viewpoint has become so entrenched that it serves as a rationale for the use of progestins in the "chemical castration" of sex-offenders.\textsuperscript{15,16} However, the physiology of P secretion reveals a marked diurnal rhythm in male rats\textsuperscript{17} and humans\textsuperscript{18-20} that positively correlates with periods of sexual behavior. Further, work with several species of reptiles has demonstrated that exogenous P, whether administered systemically or directly into the brain, will stimulate courtship and copulatory behavior in castrated males,\textsuperscript{21-25} that P is acting in its native form and not via its conversion to androgens,\textsuperscript{23} and that dihydrotestosterone (DHT) and P can synergize in stimulating sexual behavior in males\textsuperscript{22-24, 26} much as E\textsubscript{2} and P synergize in stimulating sexual behavior in female rats.\textsuperscript{27} These data prompted a reassessment of the evidence gleaned from mammalian work, and it was discovered that most of these data were derived from pharmacological dosages of P or from the administration of synthetic progestins that have anti-androgenic properties (see Reference 28).

Recent studies with rats demonstrate that P administered both systemically to produce physiological titers or directly into the POA stimulates the expression of sexual behavior of intact and castrated males\textsuperscript{29,30} and, as in the lizard studies, T and P treatments synergize to stimulate sexual behavior in castrated males.\textsuperscript{25} This work was extended recently using progesterone receptor knockout (PRKO) mice to confirm the facilitatory role of PR in sexual behavior in males. These experiments demonstrate that males with targeted disruption in the PR show a rapid loss in sexual behavior following castration relative to wildtype (WT) males and a reduced responsiveness to T replacement therapy (Figure 8.2).\textsuperscript{31} This finding parallels that observed in whiptail lizards; males sensitive to P are also sensitive to DHT.\textsuperscript{23} Finally, P may also be important in the sexual differentiation of rats. Wagner et al.\textsuperscript{32} have recently discovered that in rats the number of PR immunoreactive cells is high in the medial POA of males, but virtually absent in females, from late in gestation (embryonic day 20) until 10 days after birth. This is a period corresponding to the surge in T in males, and prenatal treatment with T will masculinize females and induce PR expression to levels similar to that of nor-
B. Evolution of the Molecular Neuroendocrine Events Regulating Sexual and Pseudo sexual Behavior

That sex steroid hormones and their receptors in the brain modulate the expression of sexual behavior is another tenet of behavioral neuroendocrinology. Although certain aspects of the structure and expression of sex steroid receptors are remarkably conserved across all vertebrate classes, sexual behaviors and their attendant physiology vary widely among species. Comparison of species with different hormone-brain-behavior relationships reveals three aspects of sex steroid receptor gene expression which may underlie species differences in endocrine physiology and behavior8 (1) neuroanatomical distribution of sex steroid receptors, (2) sensitivity to sex steroid hormones, and (3) variation in the magnitude of sex steroid hormone receptor gene expression in response to hormones.

1. The distribution of PR, AR, and ER in the brain is evolutionarily conserved. Gonadal steroid hormones act upon specific areas of the vertebrate brain to affect the reproductive physiology and behavior of the animal. Steroid receptors are members of a superfamily of transcription factors that mediate the effects of steroid hormones by modulating gene expression in the cells containing the receptors. The neuroanatomical distributions of steroid hor-
mone receptor-containing cells have been described for several species using steroid autoradiography, immunocytochemistry, and more recently, in situ hybridization. The polymerase chain reaction was used to amplify and clone fragments of the PR, AR, and the ER genes of whiptail lizards. These clones were then used to synthesize probes for use in in situ hybridization assays and to map the neuroanatomical distribution of all three sex steroid hormone receptors in the brains of parthenogenetic and sexual whiptail lizards. The distribution of receptor-containing cells in the whiptail lizard is in agreement with previous reports in other species, with receptor-containing cells concentrated in septal, amygdaloid, cortical, preoptic and hypothalamic nuclei. Studies with mammals using a variety of methods indicate that the anterior hypothalamus (AH) and preoptic area (POA) are heterogeneous structures, and the patterns of steroid receptor gene expression in the whiptail brain are consistent with this interpretation.

2. Species differences in the sensitivity to sex steroid hormones have a specific relationship with sex steroid levels and sex steroid hormone receptor expression in the brain. Circulating concentrations of gonadal steroid hormones and reproductive behavior in female vertebrates vary as a function of ovarian state. Steroids secreted by the ovary, specifically E2 and P, influence the expression of behaviors associated with reproduction by interacting with sex steroid receptors located in specific regions of the brain. Comparison of females of the ancestral sexual species to the parthenogen reveals that the circulating E2 concentrations in the parthenogen are approximately five-fold lower than in female sexual whiptails, though the display of receptive behaviors do not differ between the species. To assess whether this species difference is linked to gene expression of sex steroid receptors, ER and PR mRNA expression were analyzed in several brain regions of ovariectomized, vitellogenic, and postovulatory individuals from the sexual and unisexual species using in situ hybridization. The regulation of sex steroid receptor gene expression is region specific, and, furthermore, species differences exist in the level of sex steroid receptor gene expression in specific regions. Specifically, E2 increases the abundance of ER mRNA in the ventromedial hypothalamus (VMH) in both females of the sexual species and the parthenogen, but the magnitude of the increase is greater in the unisexual whiptail. Because of the evolutionarily conserved role of the VMH in the expression of female-like receptive behaviors, this species difference in ER mRNA expression may account for the increased sensitivity to E2 in the parthenogen.

A recent study has established that the ancestral sexual and descendant parthenogen species differ also in the estrogenic regulation of PR mRNA in the POA (Figure 8.3). While E2 treatment does not increase PR mRNA expression in the periventricular region of the POA (PvPOA) of females of the sexual species, E2 does increase PR mRNA expression in the PvPOA of the parthenogen. This finding suggests a possible proximate mechanism important in the evolution of pseudocopulatory behavior. Thus, the current model of the origin of pseudosexual behavior in the parthenogenetic whiptail lizard postulates that E2 secreted during the preovulatory phase of the ovarian cycle
FIGURE 8.3
PR mRNA abundance in the PvPOA for females of the ancestral sexual species (C. inornatus or Ci F) and the descendant parthenogenetic species (C. uniparens or Cu) given either blank or estradiol benzoate (EB) injections. Depicted is the abundance of PR mRNA measured as average number of silver grains per cluster (mean ± SEM) in the PvPOA of the ancestral sexual species and the descendant parthenogenetic species. (Redrawn from Goodwin, J. and Crews, D., Progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: Regional and species differences, J. Neurobiol., 39, 287, 1999. With permission.)

upregulates ER and PR mRNA in the VMH and stimulates the expression of female-like receptive behavior. This pattern is similar to that in females of the ancestral sexual species. The rise in E\textsubscript{2} also stimulates increases in PR mRNA in the PvPOA, sensitizing this brain region to the surge in P which follows ovulation. This report and previous work demonstrating higher PR mRNA levels in the PvPOA of the parthenogen compared to females of the sexual species over the course of the ovarian cycle suggest that a species difference in sensitivity to P in this brain region underlies the observed species difference in the display of pseudocopulatory behavior.

3. Female rats and mice, which have an abbreviated follicular phase and brief periods of estrus, differ in their reproductive physiology compared to other vertebrates, like whiptail lizards, which have extended follicular phases and prolonged periods of estrus. While much is known about how estrogens regulate sex steroid receptor expression in rats and mice, little is known about the effects of E\textsubscript{2} on sex steroid receptor expression in lizards. To better understand the molecular mechanisms involved in the control of receptive behavior in whiptail lizards, the effects of exogenous E\textsubscript{2} on the regulation of ER and PR gene expression in several brain regions were investigated. First, after determining a dosage of estradiol benzoate which reliably induced receptive behavior in ovariecto-
mized parthenogens, *in situ* hybridization was used to examine the effects of that dosage on ER and PR mRNA expression in the brain 24 h after injection. Estrogen treatment results in a significant upregulation of ER mRNA expression in the VMH, downregulation in the lateral septum, and no change in the dorsal hypothalamus and PviPOA. The same dosage results in increased PR mRNA expression in the PviPOA, but no significant changes in PR mRNA expression are observed in the periventricular nuclei of the hypothalamus or the torus semicircularis. The upregulation of ER gene expression by E$_2$ in the VMH of lizards is opposite to that reported in female rats in which E$_2$ downregulates ER expression in the ventromedial nucleus of the hypothalamus (VMN). Differences in reproductive physiology between rats and mice and other vertebrates may be related to these neural differences.

C. Sexual Dimorphisms in Behavior vs. Sexual Dimorphisms in the Hypothalamus

The large literature on sex differences in the vertebrate brain will not be reviewed here. Rather, we focus on sexual dimorphisms in the reptilian brain, and in particular in the whiptail lizard (see Reference 7). Unisexual vertebrates enable us to address from a new perspective two fundamental questions (1) Are there neural circuits for both male- and female-typical sexual behaviors? and (2) Are structural differences in brain areas related to the frequency and intensity of these behaviors?

1. Dual Neural Circuits Subserving Sexual Behavior

Are there dual neural circuits in the vertebrate brain, one mediating mounting and intromission behavior (including the AH and POA), the male-typical mating pattern, and the other mediating receptive behavior (including the VMH), the female-typical mating pattern? Although researchers have often commented on males that exhibit female-typical sexual behaviors or, conversely, females that exhibit male-typical sexual behaviors, the bulk of modern research has focused on the neuroendocrine mechanisms controlling homotypical behaviors, namely mounting behavior in gonadal males and receptive behavior in gonadal females. In other words, each neural circuit has been studied extensively, but almost always in isolation of its complement.

In males of the ancestral sexual whiptail species and in the parthenogen, intracranial implantation of androgens into the preoptic area-anterior hypothalamus (POAH) continuum elicits mounting behavior. Androgen implants into the VMH not only fail to elicit mounting behavior but also fail to affect receptive behaviors. Conversely, implantation of E$_2$ into the VMH activates receptivity both in the females of the sexual species and in the parthenogen, while E$_2$ implants into the POAH continuum have no effect on receptive or mounting behavior. Lesions of the dorsolateral VMH, an area containing high concentrations of ER mRNA in the whiptail, inhibit receptive behavior.
Lesions in the POAH continuum impair courtship both in males of the sexual species and in the parthenogen. These results highlight the conservation in vertebrates of the VMH as a brain area critical for the expression of female-typical sexual behavior and of the POAH continuum as an area integral for the expression of male-typical sexual behavior.

In the sexual whiptail the POAH is larger in males, while the VMH is larger in females. During hibernation and following castration, the POAH shrinks while the VMH enlarges (i.e., these areas become female-like). Golgi studies indicate that soma size of neurons in these areas follows the same pattern. In other words, the somata of neurons in the POAH continuum are larger in males than in females, while the somata of neurons in the VMH are larger in females. These studies indicate that sexual dimorphisms in the sexual species are seasonally plastic in the adult and sensitive to testicular androgens.

While the parthenogen displays both male-typical and female-typical sexual behaviors, its brain is not morphologically bisexual. Rather, the POAH and VMH are similar in size to those of females of the ancestral sexual species, even in parthenogens exhibiting male-typical behaviors. This same relationship holds for neuronal somata size. Even if parthenogens are treated with androgens so that they exhibit male-like copulatory behaviors and coloration, brain morphology remains unchanged and feminine. Despite this lack of apparent structural difference in these brain areas, the bisexual nature of the brain is revealed by patterns of metabolic activity. That is, 2-fluorodeoxyglucose is concentrated in the POAH of parthenogens exhibiting male-like mounting behavior and concentrated in the VMH of individuals exhibiting female-like receptive behavior. Because the parthenogens are genetically identical and of the same sex, the confounds of gender and genetic differences do not exist. Thus, the parthenogen provides a unique model for the study of neural circuits underlying sex-typical sexual behaviors in the same brain.

Aside from the structural dimorphisms between brains of male and female sexual whiptails, another dimorphic trait concerns the estrogenic regulation of ER and PR mRNA in discrete brain areas. In situ hybridization analysis has revealed sex and regional differences in estrogenic effects on ER and PR mRNA abundance in the ancestral sexual species. Females but not males respond to E₂ treatment with increases in ER and PR mRNA expression in the VMH. The VMH sex differences described here are similar to those in rats in that females exhibit estrogenic regulation of ER and PR mRNA while males do not, suggesting that this pattern is evolutionarily conserved. Neither sex nor estrogen effects have been definitively shown for ER or PR mRNA abundance in the POAH. Sex differences in the response to E₂ in the VMH may therefore underlie sex differences in the display of receptive behavior. Indeed, the parthenogen demonstrates the PR mRNA increase and displays female-like receptive behavior in response to E₂ treatment.

However, recent experiments indicate that the sexual dimorphism in the ability of the VMH to respond to E₂ is not irreversibly differentiated. In com-
parison to males castrated for 1 week, males castrated and maintained for 6 weeks will show a significant upregulation of PR mRNA in the VMH after E₂ treatment as compared to control males.⁴⁰ In fact, they do not differ from similarly treated long-term ovariectomized females, which raises the possibility that long-term castrate males might also respond behaviorally to E₂ treatment. Preliminary evidence, however, indicates this is not the case. Male whiptails are never sexually receptive to another male, even when their brains resemble those of females morphologically (as during hibernation) and in terms of steroid hormone receptor gene expression (as after long-term castration).

The expression of three sexually dimorphic behavioral and neural traits in the ancestral sexual species has been characterized. Females, but not males, will display receptive behavior in response to exogenous E₂. In addition, exogenous E₂ treatment will greatly increase PR mRNA in the VMH of females but not of males. Finally, females have smaller POAH volumes and larger VMH volumes than males,⁴⁵ and this neurophenotypic trait appears sensitive to androgen levels in males but not in females. After castration, males have POAH and VMH volumes similar to conspecific females.⁴¹ Androgen-replacement therapy reinstates male-like morphology in males, but does not masculinize neural morphology in females. The phenotype of the descendant parthenogen is very similar to that of females of the ancestral sexual species. Exogenous E₂ treatment induces receptive behavior and increases PR mRNA in the VMH of the parthenogen. The volumes of the POAH and VMH are comparable to females of the sexual species and are not affected by either ovariectomy or androgen administration. However, androgen treatment effectively induces male-typical pseudosexual behavior in the parthenogen. Thus, gross morphological changes in hypothalamic and pre-optic areas are neither necessary nor sufficient for the expression of heterotypical sexual behaviors.

Such findings raise interesting questions as to the meaning of sexual dimorphisms in the vertebrate brain. The parthenogen clearly retains the ability to express male-like behaviors. But it does so not because it has developed a morphologically masculinized POAH, but because it has co-opted the naturally-occurring P surge to trigger the masculine behavioral potential that remains in a feminized brain. Thus, research on the parthenogen suggests that behavioral differences need not be paralleled by structural differences in the brain. Indeed, structural differences do not exist in brain area volumes between courting and non-courting male whiptails during the breeding season or between courting and non-courting males castrated and given identical T treatment. Taken together these studies suggest that behavioral differences do not necessarily imply gross structural differences in the brain.
2. Genetic vs. Hormonal Organization of the Brain

A strong test of the hypothesis that structural dimorphisms in the brain need not underlie behavioral dimorphisms would be to examine “male” parthenogens. However, males have never been found in this all-female species naturally, and administration of T or DHT before and/or after hatching has consistently failed to alter ovarian development. One of us (D.C.) had come to assume that the parthenogen had lost the gene(s) required for testes development. However, it was found that if eggs are treated up to 12 days but not 20 days after oviposition with as little as 1 μg of Fadrozole, an aromatase inhibitor, all hatchlings will have fully developed testes and vasa deferentia, and lack any signs of ovarian tissue or oviducts17,42-44. This suggests that gonadal structures become irreversibly determined between embryonic Days 12 and 20, the time during which the gonad first becomes histologically distinct as an ovary in unmanipulated hatchlings. As adults, these “created males” exhibit masculinized coloration, possess male-typical accessory sex structures, and display only male-typical copulatory behaviors.

The paradigm established from studies with mammals is that the sexual differentiation of the brain is determined by hormones secreted by genetically determined differentiated gonads. Recent work in birds and mammals has suggested however that some sexually differentiated traits can be determined directly by genetic factors, independent of hormonal input.45 Specifically, the size of sexually dimorphic brain nuclei in the zebra finch46 and sexually dimorphic sensitivity of specific neurons to androgen treatment in mice47 are affected by genotype. Establishing the generality of this phenomenon across taxa is imperative, and the “created male” parthenogen enables us to address this question.

Recent studies with “created male” parthenogens indicate that sexual dimorphisms in the brain may be determined directly by genetic factors rather than by hormones. Two different measures of sexual dimorphisms in the brain, volume of the POA and estrogen regulation of PR mRNA in the VMH, are female-like in the brains of “created males,” indicating that the dimorphisms observed in the ancestral sexual species are likely due to genotype and not testicular hormones.44 Taken together, these data suggest that there is both a developmentally organized as well as genetically determined difference between male and female brains.

This work with “created males” is significant for two reasons. First, it indicates that despite the loss of males and lack of dependence upon sperm to activate development, the genes regulating testis development have been retained on the autosomes in the parthenogenetic whiptail, a finding consistent with reports that SRY, the trigger for testis development in eutherian mammals, is not specific to males in nonmammals.48,49 Second, it enables us to test in a novel way the recent hypothesis that SRY-like genes may have direct organizational effects on the brain independent of sex steroid hormones.50 The fact that male parthenogens can be created allows investigation of the independent contributions of gonadal and genetic sex. For example,
the hypothesis that estrogentic regulation of PR mRNA in the VMH is primarily controlled by gonadal sex can be rigorously tested by comparing "created male" parthenogens to unmanipulated parthenogens. Because all parthenogens are genetically identical, the influence of testicular development on sexual differentiation without the confound of genetic differences can be assessed.

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References


