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Hormones, Brain, and Behavior in Reptiles

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I. INTRODUCTION

The living group of animals we refer to as reptiles consists of at least three evolutionary lineages that encompass one of the major amniote vertebrate radiations (Fig. 1). In addition to familiar forms, such as lizards, snakes, turtles, and crocodilians, the reptiles also encompass the approximately 10,000 species of birds (Padian and Chiappe, 1998). From a general standpoint, the key value of studies of reptiles lies in two features. The first of these is the enormous diversity of patterns of sexual differentiation observed across species. The second is the presence of many primitive (in the phylogenetic sense) characters in reptiles. Among the diverse patterns represented are temperature-dependent sex determination, parthenogenesis in all-female species, and other species that exhibit distinct alternate male phenotypes.

This chapter focuses on variation in the neural substrates of sexual behavior in reptiles. We begin with a short review of diversity in sex determination, sexual differentiation, and hormone-behavior relationships observed in reptiles. This is followed by a discussion of anatomical differences, including intersexual variation in the size of neuron soma and brain nuclei and the within vs between sex differences in the function of neurons as revealed by the 2-deoxyglucose utilization and cytochrome oxidase techniques. Next, we consider within and between sex differences in the neurochemistry of brain areas subserving sexual behavior in reptiles. Reptiles show some differences in steroid receptor regulation that are similar to those described for mammals, whereas the diversity of reproductive patterns enables some comparisons not possible with mammals or birds and so extends these findings. Finally, we summarize some research directions that are likely to prove especially promising.

A. Advantages as Models

Modern reptiles exhibit phenotypes that are in many ways similar to what we assume the ancestral amniote vertebrate must have been like. These characteristics include ectothermy, oviparity, and the lack of a well-developed cerebral cortex. The presence of structures homologous to those found in mammals and birds coupled with the lack of complex cortical development characteristic of birds and mammals makes modern reptiles useful systems for examining basic behavioral controlling mechanisms in vertebrates. Such comparative research has revealed that the areas in the limbic forebrain involved in the regulation of social and sexual behaviors are ancient and conserved among vertebrates. This research has also demonstrated that differences in the distribution of sex-steroid-concentrating neurons are rare, but differences in the distribution of steroid hormone receptors and differences in the regulation of steroid hormone receptors are common. Further,

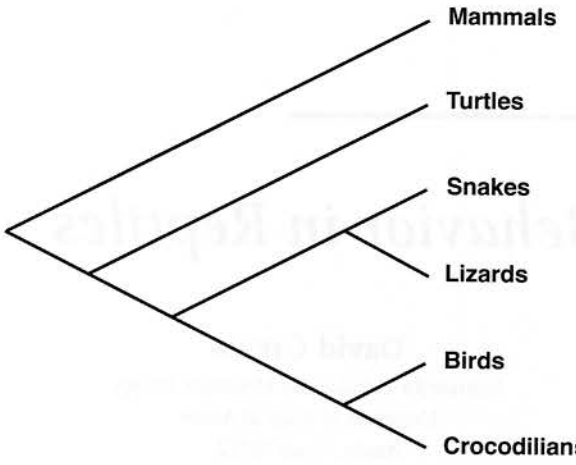

FIGURE 1 Phylogeny of amniote vertebrates. Mammals are believed to have arisen from turtle-like therapsid reptiles approximately 350 mya and modern birds from crocodilian-like archosaurs approximately 250 mya.

species differences in plasma levels of sex hormones are paralleled by differences in behavioral sensitivity to these hormones as well as by differences in the regulation of genes coding for steroid hormone receptors. Other features modern reptiles probably share with the first amniote vertebrates are mechanisms of sex determination that are variable in terms of the important cues (genotype vs environment) and in the type of genotypic sex determination that is displayed in groups (male vs female heterogamety).

This combination of diversity and conserved characters provides a variety of natural experiments with which to ask questions about basic principles of sex determination and sexual differentiation (cf. Crews and Gans, 1992). This has two consequences. First, phenomena heretofore unrecognized in other amniote groups are evident in reptiles, leading to renewed study in mammals that increases our understanding of the neuroendocrine control of sexual behavior. For example, the discovery that progesterone is important in the control of male-like pseudocopulatory behavior in parthenogenetic whiptail lizards led to studies with rats and transgenic mice that, together, revealed the importance of progesterone and its receptor in the control of male sexual behavior. Second, this diversity also allows for a variety of comparisons between species exhibiting differing patterns and for many processes, there are a sufficient of number of species, including outgroups, to generate an adequate sample size for comparisons. For example, viviparity has evolved independently from oviparity perhaps 100 or more times in reptiles (Blackburn, 1999; Guillette, 1991).

B. Pivotal Place in Amniote Evolutionary History

Reptiles as a group gave rise to two major lineages, the birds and the mammals, approximately 250 and 300 million years ago, respectively. The closest living relatives of birds are the crocodilians and their closest extinct relatives are the dinosaurs. Indeed, the skull of ratites (the ancient birds including the ostrich, emu, rhea, and kiwi) resembles that of a crocodilian in many respects. This has led to the suggestion that modern birds fall within the phylogenetic classification of reptiles. Early reptiles gave rise to today's turtles and mammals. Thus, research on the neural and endocrine control of behavior in extant reptiles help us better understand the patterns we see in birds and mammals and how these patterns may have originated.

C. Reptilian Brain as an Experimentally Tractable Model

The lack of a well-developed cortex in reptiles is experimentally advantageous in many ways. Greenberg refers to reptiles as "walking limbic systems" and notes the value of interpretations not being subject to the complications presented by a well-developed cortex (Greenberg *et al.*, 1979). Modern reptiles are probably also primitive in the neural circuitry that mediates intromission behavior and sexual receptivity. Sexual behaviors have been important for our understanding of behavioral neuroendocrinology generally and an understanding of the ancestral state of neuroendocrine mechanisms controlling these behaviors should help us better understand how they have evolved and function in birds and mammals.

II. DIVERSITY IN SEX DETERMINATION, SEXUAL DIFFERENTIATION, AND HORMONE–BEHAVIOR RELATIONSHIPS

Reptiles show an extraordinary diversity of sex determination and differentiation patterns. In addition to genotypic sex-determining mechanisms, many reptiles

possess primitive traits (e.g., temperature-dependent sex determination, TSD) or specialized traits (e.g., obligate parthenogenesis and alternative mating tactics) that have added new dimensions to our understanding of reproductive neuroendocrine mechanisms underlying reproduction in mammals. One important benefit deriving from the diversity seen in reptiles relates to studying variation in sexual behavior. Sexual behaviors and aggressive behaviors often show discontinuous variation between the sexes in birds and mammals, although there is typically considerable individual variation within the sexes (Crews, 1998). In contrast, many reptiles show more continuous variation in these behaviors. Examples include all-female species in which individuals alternate between the display of female- and male-like pseudosexual behavior during the course of the ovarian cycle, species with TSD that show substantial behavioral variation within sexes across incubation temperatures, and species that exhibit distinct alternate male phenotypes. Viewing sexuality as a continuous variable should facilitate thinking about how modern states of sexuality in birds and mammals arose. This section highlights diversity in reptilian sex determination and differentiation patterns and the research opportunities that this diversity presents.

A. Sex Determination and Sexual Differentiation in Reptiles Lacking Sex Chromosomes

Many reptiles exhibit genotypic sex determination similar to that of mammals (XX:XY) and birds (ZZ:ZW); some reptiles exhibit male heterogamety similar to mammals, whereas others exhibit female heterogamety similar to birds. However, in all crocodilians (alligators, crocodile, caiman, etc.), most turtles (all marine turtles and tortoises and many freshwater turtles), and some lizards (various Geckkonid and Agamid species), gonadal sex is established by the temperature experienced by the incubating egg (Viets *et al.*, 1994).

1. *Molecular Genetics of Temperature-Dependent Sex Determination*

Three basic patterns of TSD have been documented (Fig. 2). First, relatively high temperatures produce males, whereas relatively low temperatures produce females. The inverse pattern also exists in some species, as well as a sex-determining pattern in which intermediate temperatures produce males and high and low temperatures produce males. The sensitivity to temperature is restricted to the mid-trimester of development, the temperature-sensitive period (TSP) (Crews, 1994, 1996a). TSD is believed to be ancestral to the genotypic sex-determining pattern characteristic of birds and mammals (Crews, 1994).

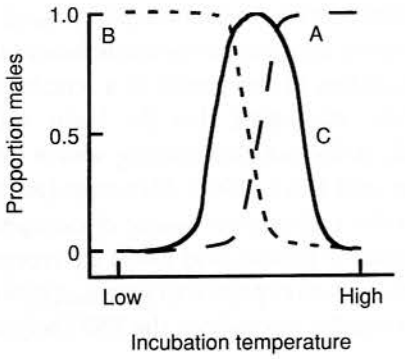

FIGURE 2 Response of hatchling sex ratio to incubation temperature in various egg-laying reptiles. These graphs represent only the approximate pattern of the response and are not drawn according to any single species. The three patterns recognized are (A) only females produced from low incubation temperatures, males at high temperatures; (B) only males produced from low incubation temperatures, females at high temperatures; and (C) only females produced at the temperature extremes, with male production at the intermediate incubation temperatures. Genotypic sex determination also occurs in reptiles with the result that the hatchling sex ratio is fixed at 1:1 despite incubation conditions.

Sex steroid hormones are implicated in the process of TSD, and estrogen, in particular, appears essential in female sex determination (Crews *et al.*, 1994, 1996a; Lance, 1997; Wibbels *et al.*, 1998). Estrogens applied exogenously to red-eared slider turtle *(Trachemys scripta)* eggs incubating at a male-producing temperature override the temperature effect and female hatchlings result (Crews *et al.*, 1991; Wibbels and Crews 1992). Exogenously applied inhibitors of aromatase override a female-producing incubation temperature, and male hatchlings result (Crews and Bergeron, 1994; Wibbels and Crews, 1994).

Research with a variety of turtle species such as the European pond turtle *(Emys orbicularis)* has shown a correlation between female incubation temperatures and increased levels of endogenous aromatase mRNA and enzyme activity in the putative ovary during the TSP (cf. Desvages and Pieau, 1992; Jeyasuria and Place,

1997, 1998). Other researchers have found that aromatase activity increases in the turtle brain prior to the time it increases in the gonad at a female-producing temperature, suggesting that the brain, rather than the gonad, is the sex-determining source of estrogen (Jeyasuria and Place, 1998; Merchant-Larios, 1998). Whatever the endogenous source of estrogen, the gonads of putative females and males are receptive to its effect because both express estrogen receptors (ERs), albeit differentially, throughout the TSP (Bergeron *et al.*, 1998).

Male sex determination can be manipulated by exogenously applied dihydrotestosterone (DHT), a nonaromatizable androgen, its derivatives, and reductase inhibitors (Wibbels *et al.*, 1992; Wibbels and Crews, 1992, 1995, Crews and Bergeron, 1994). This effect is less striking than that of estrogen in female sex determination and is only seen at intermediate, or less potent, incubation temperatures. Nevertheless, steroid hormones are undoubtedly a part of TSD in both males and females.

It is evident that the downstream events in the differentiation of the gonad in TSD reptiles and mammals and birds are similar and that both an ovary-determining cascade and a testis-determining cascade coexist in the embryo (Fig. 3). With the exception of *Sry* (in mammals) or the sex-determining gene associated with the W chromosome (in birds), the genetic cascade thus far described in the gonadal development of mammals has been identified in birds and in reptiles with TSD. For example, steroidogenic factor 1 (SF-1) is a transactivator of most enzymes involved in the biosynthesis of steroid hormones, including sex steroids (Morohashi *et al.*, 1999). SF-1 is encoded by the *ftz-f1* gene, a homolog of the *Drosophila ftz-f1* gene, and study of mice with a targeted disruption of *ftz-f1* reveals the abnormal function of pituitary gonadotropes (Morohashi *et al.*, 1999) as well as a malformed ventromedial hypothalamus (VMH) (Shinoda *et al.*, 1995; Luo *et al.*, 1994). Analysis indicates that SF-1 is expressed at the earliest stages of urogenital ridge development; in mice the disruption of the gene encoding SF-1 results in newborns that lack adrenal glands and gonads (Ikeda *et al.*, 1994; Luo *et al.*, 1994; Shen *et al.*, 1994). Both male and female embryos express SF-1; shortly after differentiation of the Sertoli cells and formation of testicular cords, SF-1 expression persists in males but diminishes in females (Luo *et al.*, 1994). In addition to being critical to sex steroid biosynthesis, SF-1, along with SOX9, up-regulates the expression of Müllerian inhibiting substance (MIS) in the Sertoli cells of developing testes and the MIS receptor in testes and Müllerian ducts (Arango *et al.*, 1999). MIS in turn appears to down-regulate aromatase gene expression (DiClemente *et al.*, 1992; Rouiller-Fabre *et al.*, 1998). A brain-specific transcript of aromatase has been detected in rats, and the gene encoding it contains a consensus SF-1 binding site (Honda *et al.*, 1994). In reptiles and birds, it is thought that SF-1 might regulate aromatase, and hence the synthesis of ovary-determining estrogen, in developing ovaries (Fleming *et al.*, 1999; Western *et al.*, 2000; Smith *et al.*, 1999).

The pattern of SF-1 expression in the chicken and alligator following histological distinction of gonadal sex differs from that in mammals. As gonadal sex becomes distinct, SF-1 levels become less abundant in the genetically or temperature-determined male than in the female chicken (Smith *et al.*, 1999) and alligator (Western *et al.*, 2000), respectively. In the chicken, SF-1 message expression falls to an almost negligible level in males but remains high in females, correlating with the pattern of aromatase expression in chickens (Andrews *et al.*, 1997; Smith *et al.*, 1999). Aromatase is up-regulated by SF-1 in mammalian granulosa cells (Carlone and Richards, 1997), where it converts testosterone (T) to estradiol (E_2).

Studies of the expression of SF-1 mRNA in the red-eared slider turtle, a species with TSD, reveals that SF-1 is equivalent at male- and female-producing temperatures in the early gonadal ridge (Fleming *et al.*, 1999). At this stage, gonads from the two incubation temperatures are bipotential and histologically indistinguishable. As the gonads become histologically distinct, the pattern of SF-1 expression changes, continuing to increase at the male-producing incubation temperature but declining at the female-producing incubation temperature. SF-1 mRNA is also evident in the brain. Shifting eggs from a male- to a female-producing incubation temperature—or vice versa—at the middle of the TSP results in the down-regulation of SF-1 message levels to a female level (Fleming and Crews, 2000). The opposite pattern is observed when eggs are shifted from a female- to a male-producing incubation temperature; that is, there is an up-regulation of SF-1 mRNA levels.

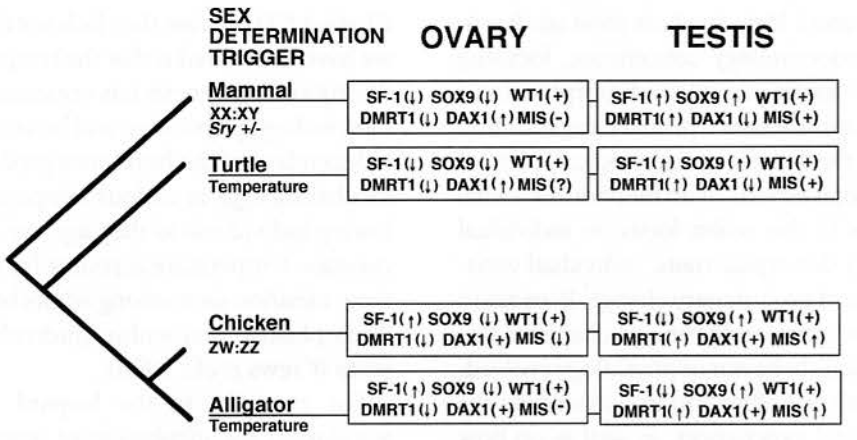

FIGURE 3 Selected genes underlying differentiation of the genital ridge into an ovary (upper panel) or testis (lower panel) in amniote vertebrates. Phylogenetic relationship of different groups is indicated at the left. In mammals and birds, gonadal sex is established by the genetic composition inherited at fertilization, a process known as genotypic sex determination (GSD). In some reptiles, gonadal sex depends ultimately on the temperature of the incubating egg, a process known as temperature- dependent sex determination (TSD). The trigger for gonad determination in mammals is the presence (or absence) of *Sry;* in birds the trigger is unknown but appears to be the ratio of the Z chromosome and autosomes. Note that many of the same genes appear to be involved in gonadal differentiation for species exhibiting GSD (mammals and birds) and TSD (turtles and crocodilians). Note also that for these selected genes the patterns of expression appear to reflect phylogenetic relationships, with mammals being similar to turtles, and birds more similar to crocodilians. The regulatory mechanisms behind the expression patterns for most of these selected genes are being investigated, but timing of SOX9 and MIS expression during testis development appears to fall along phylogenetic lines; in mammals and turtles SOX9 expression precedes MIS expression, whereas in alligator and bird the reverse pattern is seen. Finally, by manipulating the genetic, physical, or chemical environment in the various amniote vertebrates it is possible to modify gonadal sex in both GSD and TSD amniote vertebrates. DAX1, dosage-sensitive sex-reversal adrenal hypoplasia congenital critical region on the X chromosome; DMRT1, DM-related transcription factor one; MIS, Müllerian inhibiting substance; SF-1, steroidogenic factor one; SOX9, SRY-related HMG box nine; SRY, Sex-determining region on the Y chromosome; WT1, Wilm's tumor one. Plus (+) indicates presence and minus (−) indicates absence. Up arrow (↑) indicates up-regulation and down arrow (↓) indicates down-regulation.

Gonadal sex in the red-eared slider turtle and other reptiles with TSP can also be manipulated during the TSP by treating eggs incubating at a male-producing temperature with E_2 or by treating eggs incubating at a female-producing temperature with an aromatase inhibitor. Such manipulations produce female and male offspring, respectively. Following E_2 treatment of eggs incubating at a male-producing temperature, gonadal SF-1 expression is down-regulated and becomes statistically and histologically indistinguishable from temperature-derived females. The treatment of eggs incubating at a female-biased temperature with aromatase inhibitor has the opposite effect.

2. *Organizing Influence of Incubation Temperature*

There are two levels in the organization of sexuality (Crews, 1998b). Primary organization refers to the process of differentiation of the primary and secondary structures (gonads and associated duct systems) and accessory sex characters (various glands and

morphological features). Here is where most studies in behavioral neuroendocrinology concentrate, focusing on group mean differences in sexually dimorphic traits. Secondary organization follows primary organization and is manifest as the unique morphological, physiological, and behavioral aspects of an individual's sexual phenotype. Studies in this realm focus on individual variation in sexually dimorphic traits. Individual variation is the substance of evolutionary change. If we are to understand how the neuroendocrine mechanisms underlying an individual's behavior or physiology evolved, we must concentrate our efforts on understanding the prenatal and postnatal experiences, as well as on how stimuli arising from the abiotic and biotic environment influence the development of the individual (Crews, 1999). This requires both new paradigms and animal model systems that allow the separation of the effects of genes and hormones from environmental and experiential stimuli. For example, the great majority of the research on the proximate mechanisms underlying sexually dimorphic traits has emphasized the role of gonadal sex hormones. Further, this work almost invariably has used species in which gonadal sex is determined by sex chromosomes. In such species, genetic sex and gonadal sex, and hence the nature and pattern of hormones produced, are linked, making it difficult to distinguish environmental from genetic and hormonal contributions to individual differences.

Animals with environmental sex determination, such as lizards with TSD, are particularly suitable for this type of work. The leopard gecko, in particular, has proven to be an excellent model system for several reasons. First, the investigator has precise control of the critical environmental variables (in this case, incubation temperature) that determine gonadal sex and, hence, its products. Thus, by comparing males and females at certain incubation temperatures it is possible to determine the effect of gonadal sex steroids on sexually dimorphic aspects of the nervous system. Comparing same-sex individuals across a range of incubation temperatures, on the other hand, can assess individual differences among males and females. This is analogous to comparing males and females from different intrauterine positions in rodents (see Chapter 68 in Volume 4) and alternative sexual phenotypes in genotypic sex determination (GSD) reptiles (see later). Finally, these animals eliminate possible sex-specific genetic effects (see Chapter 63) because they lack sex chromosomes. What we have discovered is that the temperature experienced during embryogenesis has organizational effects on the morphology, physiology, and behavior of adults that are independent of the hormones produced by gonads. By incubating eggs at various temperatures and then following individuals as they age, we have found that incubation temperature accounts for much of the phenotypic variation seen among adults both between (sexual dimorphisms) and within (individual differences) the sexes (Crews *et al.*, 1998).

For example, in the leopard gecko (*Eublepharis macularius*) the incubation of eggs at 26°C produces only female hatchlings, incubation at 30°C produces a female-biased sex ratio, and incubation at 32.5°C produces a male-biased sex ratio; incubation of 34–35°C again produces virtually all females (Fig. 4). Hence, females from eggs incubated at 26°C are referred to

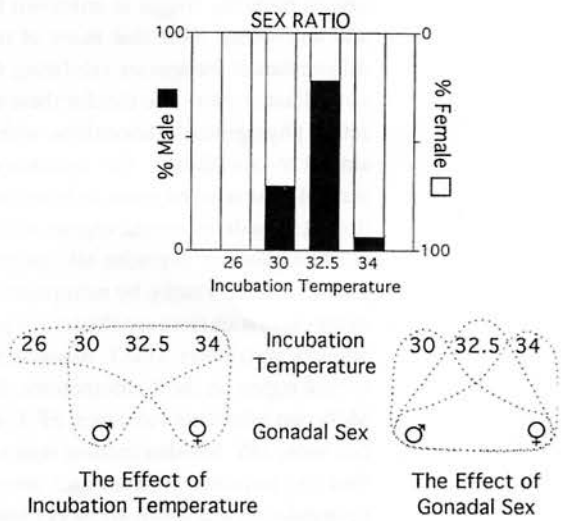

FIGURE 4 Pattern of temperature-dependent sex determination in the leopard gecko. (A) Effect of incubation temperature on sex ratio. Extreme temperatures produce females, whereas intermediate temperatures produce different ratios. Because the effects of incubation temperature and gonadal sex covary, any difference between individuals could be due to the incubation temperature of the egg, the gonadal sex of the individual, or both factors combined. To assess the contribution of each, they must be dissociated. (B) Studying same-sex animals that differ only in the incubation temperature experienced reveals the effects of temperature. (C) Comparing males and females from the same incubation temperature reveals the effects of gonadal sex. Dotted lines group comparisons made in each condition.

as low-temperature females and females from eggs incubated at 34°C are referred to as high-temperature females; the two intermediate incubation temperatures are referred to as female-biased (30°C) and male-biased (32.5°C) temperatures. Adult leopard geckos are sexually dimorphic, with males having open secretory pores anterior to the cloaca. In low-temperature females these pores are closed, whereas in females from a male-biased temperature they are open (Gutzke and Crews, 1988). Head size is also sexually dimorphic, with males having wider heads than females; yet in females, those from a male-biased temperature have wider heads than do those from a low temperature (Gutzke and Crews, 1988). Similarly, although males are the larger sex, incubation temperature has a marked effect on growth within a sex. Females from a male-biased temperature grow faster and larger than do females from female-biased temperatures and become as large as males from female-biased temperatures (Tousignant and Crews, 1995). Indeed, female geckos from estrogen-treated eggs incubated at a male-biased temperature (which overcomes the male-determining temperature effect and produces females) do not differ in growth rates from unmanipulated females from the same temperature.

Circulating concentrations of T in both newborn and adult males are approximately 100 times higher than in adult females (Table 1) (Gutzke and Crews, 1988; Tousignant and Crews, 1995). However, the endocrine physiology of the adult varies in part due to the temperature experienced during incubation (Fig. 5) (Coomber *et al.*, 1997; Tousignant *et al.*, 1995). For example, plasma estrogen levels are significantly higher in males from female-biased temperatures than in males from a male-biased temperature. Circulating estrogen levels are significantly higher and androgen levels are significantly lower in low-temperature females than in females from a male-biased temperature. Although circulating concentrations of sex hormones do differ between male and female hatchlings (Table 1), whether this also is the case in hatchlings from different incubation temperatures is not known.

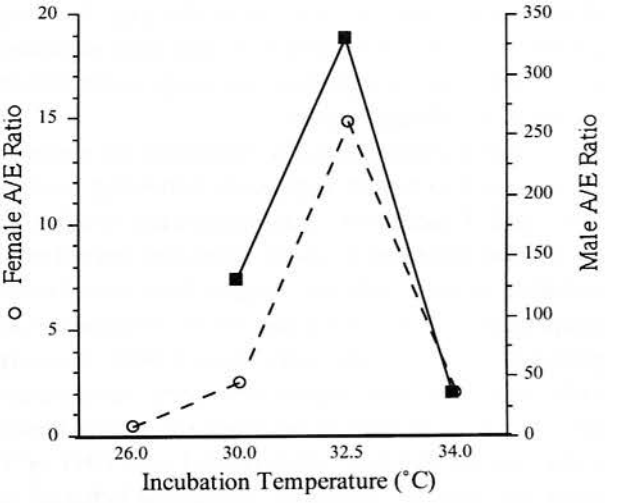

FIGURE 5 Ratio of the plasma levels of total androgens (A) and estrogens (E) in adult female (dashed line) and male (solid line) leopard geckos *(Eublepharis macularius)* from different incubation temperatures. Data from Coomber *et al.* (1997); Gutzke and Crews (1988).

Incubation temperature also has a major influence on the nature and frequency of the behavior displayed by the adult leopard gecko. Females usually respond aggressively only if attacked, whereas males posture and then attack other males, but rarely females (Gutzke and Crews, 1988; Flores *et al.*, 1994). However, males from a female-biased temperature are less aggressive than males from the higher male-biased temperature and, although not as aggressive as males from that same incubation temperature, females from a male-biased temperature are significantly more aggressive toward males than are females from a low or female-biased temperature. These same females show the male-typical pattern

TABLE 1
Circulating Concentrations of Steroid Hormones in Hatchling and Adult Leopard Geckos, Female-Biased Incubation Temperature (30°C)

Sex	*Number*	*Testosterone*	*Estradiol-17β*
Hatchlings			
Male	5	1.43 (.70)	0.14 (.03)
Female	6	0.20 (.02)	0.12 (.02)
Adults			
Male	6	77.92 (26.40)	0.48 (.06)
Female	7	1.23 (.45)	0.49 (.05)

[a]Mean concentration (ng/ml) is presented; standard error in parentheses. For hatchlings, due to the small blood volumes of hatchlings, samples were pooled ($n = 2$–6 hatchlings/pool) and number indicates number of pooled samples. For adults, number indicates individual animals.

of offensive aggression and, as is the case for body growth, females from estrogen-treated eggs incubated at the male-biased temperature are as aggressive as their unmanipulated counterparts.

Incubation temperature also influences the ability of exogenous T to restore aggression. Following ovariectomy and T treatment, low-temperature females do not exhibit increased levels of aggression toward male stimulus animals, whereas females from male-biased temperatures return to the high levels exhibited while gonadally intact (Flores and Crews, 1995). Similarly, males from the male-biased embryonic temperature scent mark more than males from the female-biased embryonic temperature when treated with DHT or T; treatment with E_2, decreases submissive behavior in males from a male-biased embryonic temperature compared to males from a female-biased embryonic temperature (Rhen and Crews, 1999). Such data suggest that incubation temperature influences how the individual responds to steroid hormones in adulthood.

Courtship is a male-typical behavior. In a sexual encounter, the male slowly approaches the female, touching the substrate or licking the air with his tongue. Males also have a characteristic tail vibration, creating a buzzing sound, when they detect a female. Intact females have never been observed to exhibit this tail-vibration behavior, regardless of their incubation temperature. However, if ovariectomized females from low and male-biased temperatures are treated with T, they begin to tail-vibrate toward female, but not male, stimulus animals; males appear to regard such females as male because they are attacked (Flores and Crews, 1995).

Attractiveness is a female-typical trait and is measured by the intensity of a sexually active male's courtship behavior toward the female. Females from a male-biased temperature are less attractive than are females from lower incubation temperatures (Flores *et al.*, 1994). Interestingly, attractiveness in high-temperature females is greater than that of females from male-biased temperatures and not different from that of low-temperature females. Long-term castrated males are attractive and initially courted by intact males, but on olfactory inspection they are attacked. This suggests that both sexes can produce both a female-typical attractiveness pheromone and a male-typical recognition pheromone, as does the red-sided garter snake (Mason *et al.*, 1989). As is the case in females, incubation temperature influences sensitivity to exogenous hormones in males. Estrogen treatment induces receptive behavior in castrated males if they were incubated at a female-biased temperature, but not if they were incubated at a male-biased temperature.

As might be predicted, these behavioral differences among and between male and female leopard geckos from different incubation temperatures also reflect differences in the neural substrates regulating these behaviors, including the size and metabolic activity of different limbic areas (see later).

3. *Parthenogenesis*

Another variation in sex determination found in reptiles is obligate parthenogenesis. In three families of lizard (teiid, agamid, and lacertid lizards) there are species that consist only of females that reproduce by cloning. Among the whiptail lizards (*Cnemidophorus* spp.) fully one-third of the species reproduce by obligate parthenogenesis. The best studied to date is the triploid *Cnemidophorus uniparens*. This particular species arose from the hybrid mating between two sexual species, and restriction analysis of mitochondrial DNA indicates that two-thirds of its genome comes from *C. inornatus* (Densmore *et al.*, 1989) (Fig. 6).

Parthenogenesis in *C. uniparens* is accompanied by a fascinating reproductive adaptation—females display male-like pseudocopulatory behavior that is indistinguishable from the male-typical courtship and mounting behavior seen in males of their direct sexual ancestor, *C. inornatus* (Crews and Fitzgerald, 1980) (Fig. 7). This behavior functions to facilitate reproduction among the females, much like male courtship serves to stimulate and synchronize reproductive activity in conspecific females (Crews *et al.*, 1980). Indeed, the process is fundamental to reproduction in all living organisms, including various forms in which males do not exist (Crews, 1996b, 1998a). The display of pseudosexual behavior is associated predominantly with the postovulatory phase of the ovarian cycle when progesterone (P) levels are elevated (Moore *et al.*, 1985a,b). The P sensitivity of male-like pseudosexual behavior in *C. uniparens* has an evolutionary antecedent in *C. inornatus*, in which P acting alone and in synergism with androgens stimulates male-typical mounting and intromission behavior (Lindzey and Crews, 1992).

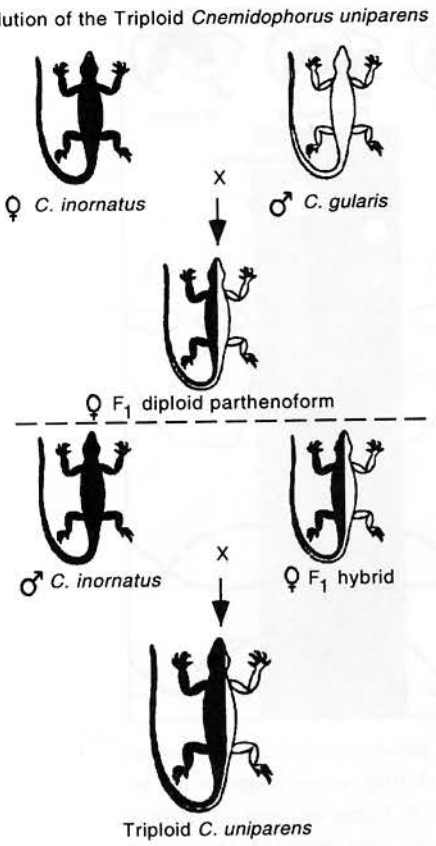

FIGURE 6 Most whiptail lizard species (genus *Cnemidophorus*) are gonochoristic, having both male and female individuals that reproduce sexually. However, one- third of the 45 species of whiptail lizards are unisexual, consisting only of individuals that reproduce by true parthenogenesis. The parthenogenetic species arose fully formed from the hybrid mating of two sexual whiptail species. Indeed, in many instances, we know which species were involved. The best-studied parthenogen is *C. uniparens*. The maternal ancestor of *C. uniparens* is the Little Striped Whiptail, *C. inornatus*. The paternal ancestor is still under dispute, with some favoring *C. gularis* and others favoring *C. burti*. Whatever the paternal species, it is known that *C. uniparens* arose from the F1 hybrid mating in a backcross with *C. inornatus*.

This discovery of P sensitivity in male lizards led to a revisitation of the question and the demonstration of similar sensitivity in male rats (Witt *et al.*, 1995).

B. Functional Associations in Hormones, Gamete Production, and Mating Behavior

Species that evolved under different constraints presumably exhibit different patterns of reproduction and therefore are likely to have fundamentally different neuroendocrine mechanisms controlling their reproduction and associated behaviors. The display of reproductive behavior in reptiles and other vertebrate groups shows one of three basic temporal relationships to gamete production (Fig. 8). The most common relationship is an associated reproductive pattern. Animals displaying this pattern exhibit sexual behavior when their gonads are actively producing gametes and steroid hormone levels are elevated. Reptilian examples of this pattern are the green anole lizard *(Anolis carolinensis)*, the sea turtles discussed in the next part of this section, and many of the other species discussed in this chapter. The display of mating behavior may also be temporally uncoupled from gamete production. This is a dissociated reproductive pattern. The most thoroughly reptilian example of this pattern remains the red-sided garter snake, *Thamnophis sirtalis parietalis* (Crews, 1983). This species is discussed at length later. The third possible temporal relationship between gametogenic activity and reproductive behavior is a constant reproductive pattern. This pattern is characterized by the maintenance of reproductive readiness (mature sperm and ovarian follicles), but with actual reproduction and the display of associated behaviors limited to typically short and unpredictable periods when environmental conditions are such that reproduction can be successful. Although this pattern has not been documented in any reptile, it does characterize wild populations of zebra finches in unpredictable desert environments (Sossinka, 1980; Allen and Hume, 1997) and remains a possibility for reptiles facing similar challenges.

This prediction that the wide variety of neuroendocrine mechanisms that subserve sexual behavior observed among vertebrate animals arose as adaptations to various ecological, phylogenetic, developmental, and physiological constraints has been borne out. Carefully chosen comparisons have led to some unexpected outcomes and insights. For example, there is a tendency to assume that there is a fundamental functional association among the three basic components of vertebrate reproduction—gametes, steroid hormones, and behavior (Fig. 9). Beginning with studies of reptiles (Crews *et al.*, 1984) and extending to other vertebrates (Crews, 1987), it has become clear that there is no intrinsic linkage between the production of gametes, the secretion of gonadal steroid hormones, and the expression

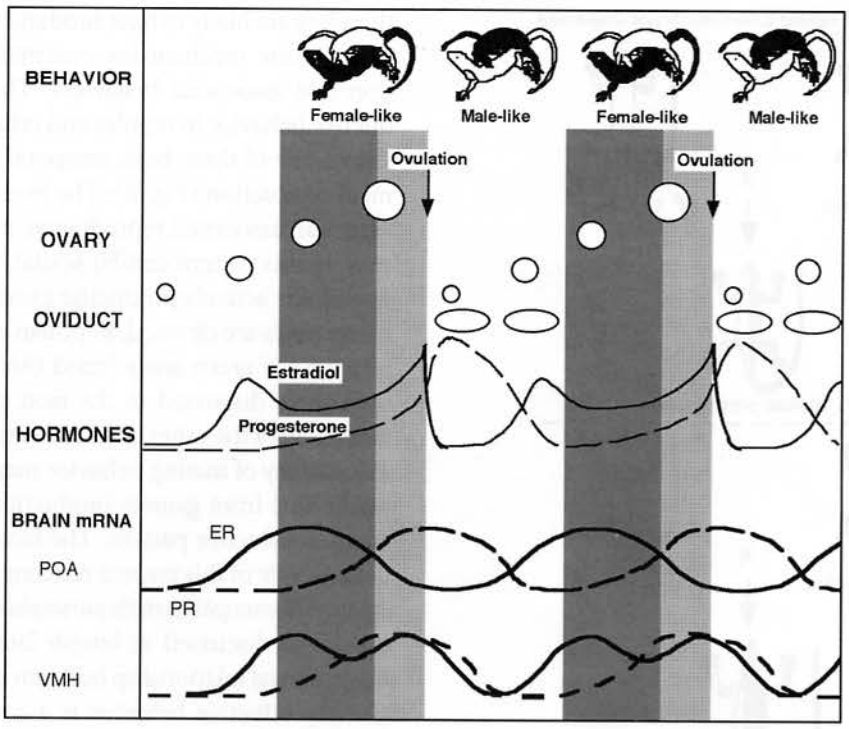

FIGURE 7 Relation among male-like and female-like pseudosexual behavior, ovarian state, and circulating levels of E_2 and progesterone (P) during various stages of the reproductive cycle of the parthenogenetic whiptail lizard. The transition from receptive to mounting behavior occurs at the time of ovulation (arrow). Also shown are the relative changes in abundance of the gene transcripts coding for ERs and progesterone receptors (PR) in the preoptic area (POA) and the VMH, brain areas that are involved in the regulation of male- and female-typical sexual behaviors. Redrawn from Crews *et al.*, 1998a.

of sexual behavior. Indeed, of the six relationships possible among these three elements, only one can be regarded as fundamental, namely that gametes cannot be produced independent of steroid hormone secretion (Crews *et al.*, 1984; Crews, 1987). A number of studies now have shown that sexual behavior need not depend on increased levels of sex steroid hormones and, further, that males and females of a particular species may regulate similar reproductive (behavioral) events by using different proximate cues and mechanisms.

C. Alternative Mating Strategies

Many species of reptiles show discrete alternate male phenotypes that exhibit discontinuous variation in male morphology, physiology, and behavior. Alternative male phenotypes are also found in other vertebrate groups, including fishes, amphibians, birds, and mammals, as well as in many invertebrates. Often one male phenotype in these systems is similar to females and the other shows exaggerated male characters. These systems have the advantage of allowing comparisons of differing behavioral phenotypes without the confound of a difference in gonadal sex (Moore, 1991). For this reason, it has been suggested that alternate reproductive phenotypes present a valuable opportunity to explore the physiological and neural mechanisms underlying individual variation in behavior and morphology. Males and females also represent alternate reproductive phenotypes, but sex comparisons face an important confounding factor—the groups being compared differ in both behavior and the type of gonad they possess. Moore (1991) proposed that alternate reproductive phenotypes within a sex avoid this complication (because the groups being compared do not differ in

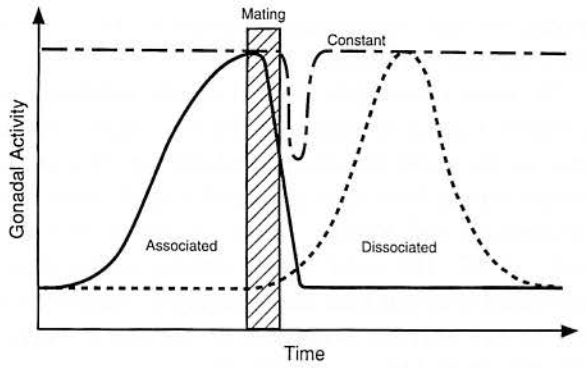

FIGURE 8 Vertebrates display a variety of reproductive patterns. Here gonadal activity is defined as the development of eggs and sperm or increased sex-steroid-hormone secretion. Individuals exhibiting the associated reproductive pattern (solid line) live in temperate regions where seasonal cycles are regular and prolonged; in such species, the gonads are fully developed at the time of mating and circulating levels of sex hormones are maximal. Individuals exhibiting the dissociated reproductive pattern (dashed line) live in extreme environments in which seasonal changes are regular, but the length of time available for breeding is limited; in such species, the gonads are small and sex-steroid-hormone levels are low at the time of mating. Individuals exhibiting a constant reproductive pattern (hatched line) live in harsh environments where breeding conditions are completely unpredictable; in such species, the gonads are maintained at nearly maximal development so that when breeding conditions do arise, breeding can occur immediately. Just as the reproductive cycles have adapted to the environment, so too have the neuroendocrine mechanisms subserving breeding behavior. The temporal uncoupling of sexual behavior and gonadal recrudescence in vertebrates exhibiting these different reproductive patterns is reflected in the dynamics of their hormone–brain–behavior relationship. The dimension of reproductive pattern is depicted here as mutually exclusive extremes only for the sake of argument; intermediate forms are known to exist.

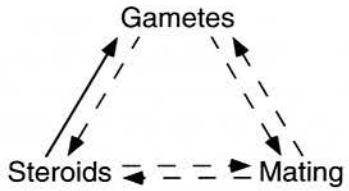

FIGURE 9 The three major components of the reproductive process in vertebrates. In species in which the pattern of gonadal activity is associated temporally with mating, as occurs in many mammals and birds, these elements are functionally associated. This has led to the paradigm that mating behavior is activated by increasing levels of gonadal steroid hormones in the circulation. However, there is no intrinsic functional association among these elements. Indeed, studies indicate that the dependence of mating behavior on sex hormones depends on the reproductive pattern exhibited, which in turn depends on various ecological, phylogenetc, developmental, and physiological constraints. The solid line indicates the only functional association shared by all living vertebrates, indicating that it is the primitive or ancient characteristic. The dashed lines represent the various functional associations observed in vertebrates. Whether these are derived associations that evolved independently in the different genera, and therefore are analogous, or they were present in the common ancestor of each Class (e.g., mammals) and therefore are homologous can only be determined through comparative analysis.

type of gonad) and are therefore valuable models for exploring the bases of ubiquitous individual variation within the sexes.

In order for this approach to be a useful one conceptually and operationally, the physiological mechanisms operating to generate differences in behavior and morphology between alternate within-sex phenotypes should be similar to those documented to produce between-sex differences. The organizational concept proposed by Phoenix and coworkers (1959) has provided the most valuable framework for understanding sex differences and has been applied to understanding the endocrine bases of alternate male phenotypes (reviewed in Moore *et al.*, 1998; see later), although the applicability of this model to other variations in vertebrate sexual determination and differentiation has been questioned (Crews, 1993).

The behavioral and ecological correlates of alternative reproductive phenotypes in reptiles are better explored than their endocrine bases so far. The hormonal bases of these alternate phenotypes have been addressed in only a few species of reptiles and the neural correlates of behavioral differences in these species have received little detailed study. However, studies in fishes that display alternate male phenotypes suggest that this will be a productive area of research in reptiles (see Grober and Bass, Chapter 23). Moore (1991) proposed the relative plasticity hypothesis as a basis for understanding the diversity of behavioral and morphological expression observed with alternate male phenotypes. Briefly, this hypothesis proposes that fixed differences between alternate phenotypes are due to organizational actions of steroid hormones, whereas more plastic differences are due to activational

influences of these hormones. Moore, Hews, and Knapp (1998) refined this hypothesis to account for cases in which permanent phenotypic effects might require the actions of the relevant hormones only during critical developmental windows.

We briefly review two cases here for which physiological information is available regarding alternative reproductive phenotypes in reptiles. We also consider a genus of iguanid lizards (*Sceloporus* spp.) in which variation in male phenotypes is common both in and across closely related species. The best-characterized models of alternate reproductive phenotype variation from a physiological perspective include the red-sided garter snake (*Thamnophis sirtalis parietalis*) and the tree lizard (*Urosaurus ornatus*) (reviewed later). There are a number of other reptiles displaying alternate reproductive phenotypes whose behavior and ecology are becoming well understood, but for which the endocrinology and neurobiology of behavioral variation have not been studied. These promising models include the side-blotched lizard (*Uta stansburiana*) (e.g., Sinervo and Lively, 1996) and a variety of *Sceloporus* species (see later).

The first physiological studies exploring alternate male phenotypes focused on the red-sided garter snake. As previously described, this species exhibits a reproductive pattern in which peak levels of gonadal hormones are temporally dissociated from the display of reproductive behavior (Crews, 1991). Males emerge from winter hibernacula before females and vigorously court and attempt copulations in multimale mating balls as females emerge (Crews, 1983). Two pheromones underlie male courtship behavior (Mason *et al.*, 1989). The first is an estrogen-dependent attractivity pheromone produced by females that elicits vigorous courtship from males. The second pheromone, which most males produce, identifies them as males and hence not the object of courtship. However, a small proportion of males (termed she-males) actually produces the attractivity pheromone that characterizes females (Mason and Crews, 1985). This female mimicry serves to confuse other males in mating aggregations and appears to increase the chances of successful mating by the she-males. She-males have higher circulating concentrations of T (Mason and Crews, 1985) and a greater abundance of aromatase in the skin (Krohmer, 1989), which presumably converts the endogenous T to estrogen, thereby stimulating production of the female attractiveness pheromone.

The most thoroughly studied reptile exhibiting alternative mating strategies is the tree lizard. In this species, the males possess colored dewlaps that are extended during both aggressive and sexual interactions (Thompson and Moore, 1991a; reviewed in Moore *et al.*, 1998). The color of this dewlap varies among males and shows at least nine geographic variants with one to five variants occurring in any given location (Thompson and Moore, 1991b). Experiments in which males are raised in a common laboratory environment indicate that the basis of this dewlap color variation is either genetic or maternal in origin because the orange and orange-blue phenotypes develop in approximately the same proportions as are observed in the wild source populations (Thompson *et al.*, 1993; Hews *et al.*, 1997). Most attention has focused on one Arizona location in which two male morphs exist—one with orange-blue dewlaps (orange-blue males) that is site attached and holds territories encompassing the territories of three to four females and one with orange dewlaps (orange males) that is more nomadic under poor habitat conditions (i.e., in drought years) and sedentary with small home ranges under good habitat conditions. Orange-blue males are more aggressive both in the laboratory and in nature (Thompson and Moore, 1991a, 1992).

The effects of gonadal steroid and glucocorticoid hormones in male tree lizards support the predictions of Moore's relative plasticity hypothesis. Adult T and corticosterone levels do not differ between orange-blue and orange males, and neither castration nor androgen manipulations alter dewlap color expression in adult males (Moore *et al.*, 1998). In contrast, the castration of neonatal male tree lizards increases the proportion developing into orange males, whereas T implants given on hatching or 30 days thereafter increase the proportion developing into orange-blue males (Hews *et al.*, 1994) (Fig. 10). By 60 days posthatching, T implants are ineffective in altering dewlap color, indicating a defined early critical period for the development of this trait, as has been shown for behavioral organization by steroid hormones in mammals. Patterns of plasma androgens in developing male tree lizards do suggest a possible bimodality in male androgen levels during the period in which dewlap color develops (Moore *et al.*,

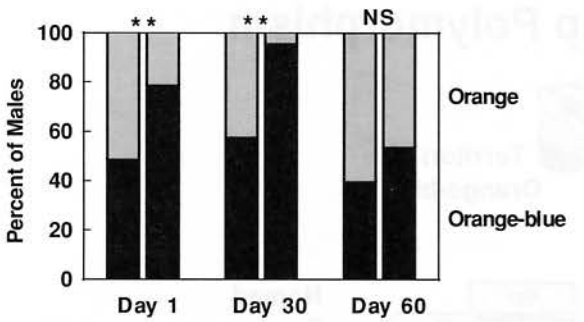

FIGURE 10 Testosterone manipulations of hatchlings alter adult dewlap coloration (day 90) in male tree lizards. This figure shows the results of T manipulation experiments begun at different times following hatching. The left bar of each pair represents morph frequency in control males who did not receive T, and the right bar depicts the frequency of different morphs for males receiving T implants. Significant differences are indicated by asterisks; NS indicates no significant difference was found between the groups. Redrawn from Moore *et al.* (1998).

1998), but much more striking is a clear bimodality in P levels on the day of hatching. This possible role for P in determining morph type has experimental support. Single injections of P on the day of hatching significantly increased the proportion of males developing as orange-blue males (Moore *et al.*, 1998). This organizational role for P may point to an unrecognized developmental role for this steroid in other vertebrate systems. It is also reminiscent of the important role played by P in stimulating male-typical sexual behavior and male-like pseudosexual behaviors in whiptail lizards, as already discussed.

Corticosteroids play important roles in short-term behavioral responses in male tree lizards, supporting the relative plasticity hypothesis and adding to a growing body of information indicating these steroids are important mediators of behavioral plasticity. Corticosterone levels vary in both orange and orange-blue males depending on habitat conditions, being higher in dry years (Moore *et al.*, 1998). In the aggressive orange-blue males, corticosterone levels are temporarily higher in losers of long-term laboratory dominance interactions, but show the opposite pattern in winners of short-duration encounters (Knapp and Moore, 1995). In the field, the less aggressive orange males show both less intense aggressive behavior and greater corticosterone elevations in response to an aggressive encounter than do orange-blue males (Knapp and Moore, 1996). Males of the two morphs also differ in their response to exogenous corticosterone. Both morphs show decreases in circulating T, but this decrease is greater in the subordinate orange males. Knapp and Moore (1997) hypothesize that the greater sensitivity of T levels to elevations in corticosterone in subordinate orange males accounts for the fact that these males switch between roving and sedentary satellite patterns of space use depending on habitat conditions, whereas changes in space use are not seen in the more aggressive orange-blue males (Fig. 11).

The work on the tree lizard is supported by studies in the side-blotched lizard *(U. stansburiana)*. This species has three male morphs that are distinct in both morphological characteristics and behavior (Sinervo and Lively, 1996). Unlike tree lizards, there is some plasticity in male morph type in side-blotched lizards, in that the female-mimic yellow-throated males can become mate-guarding blue-throated males, but not the ultradominant orange-throated morph. The orange-throated morph has higher plasma levels of T, lower year-to-year survivorship, greater endurance, higher activity generally, and a larger home range that overlaps the areas used by more females than either the yellow- or blue-throated males (Sinervo *et al.*, 2000). A series of studies have shown that corticosterone decreases aggression by adult males in aquaria (Denardo and Licht, 1993) and both home-range size and activity levels in the field (Denardo and Sinervo, 1994a), even when corticosterone is given in combination with T. This effect of corticosterone on male home range appears to depend on interactions with neighboring males (Denardo and Sinervo, 1994b). Corticosterone-implanted males decreased home-range size if some neighboring males were saline-implanted, but not if all neighboring males received corticosterone implants. Testosterone implants can increase home-range size in male side-blotched lizards if not given with corticosterone (Denardo and Sinervo, 1994b). Experimentally elevating T levels in yellow- and blue-throated males to those found in orange-throated males also increases both endurance and access to females in nature (Sinervo *et al.*, 2000).

Although not as well studied from the standpoint of endocrine physiology, other groups of reptiles present opportunities for exploring the mechanisms underlying behavioral variation in and across species. One particularly promising group is the speciose lizard

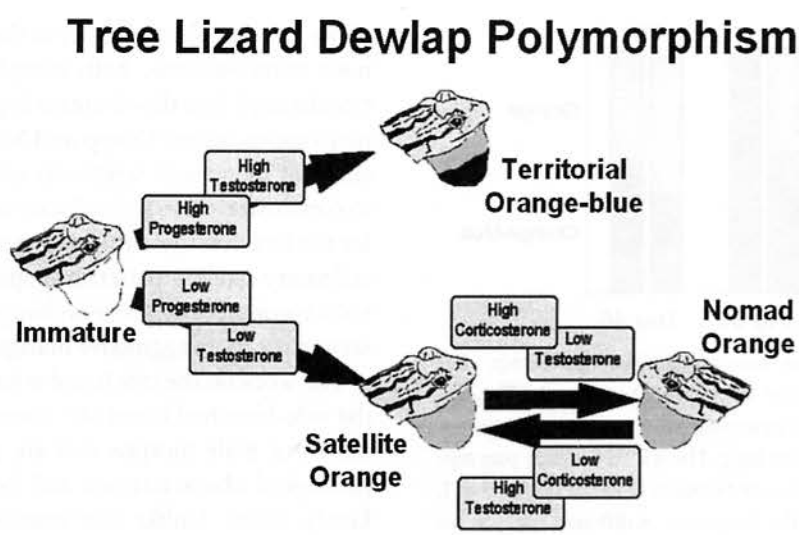

FIGURE 11 Moore and coworkers' model for organizational and activational influences on male phenotype development in tree lizards. Both testosterone and progesterone act during early development to affect adult dewlap coloration. Plastic switches between satellite and nomadic tactics in adult males of the less-aggressive orange morph are hypothesized to be due to an interplay of testosterone and corticosterone. Redrawn from Moore *et al.* (1998).

genus *Sceloporus*. There is a large body of behavioral and ecological information for *Sceloporus* species and both phylogenetic relationships in the genus as a whole (Wiens, 1993), and the evolution of dimorphic coloration and behavior in and across species have received attention (Wiens *et al.*, 1999; Wiens, 2000). Finally, there is a growing body of information on the endocrine bases of sexual phenotype development and the hormonal consequences of social interactions in this genus.

As with alternate male types in the tree lizard, various *Sceloporus* species show geographic variation in male coloration (Rand, 1990; Wiens *et al.*, 1999). There are also effects of steroid hormones on coloration in *Sceloporus*. Both orange facial color and a blue ventral coloration are influenced by T implants in adult red-lipped western fence lizards *(Sceloporus undulatus erythrocheilus)*, with effects on ventral coloration being greater in males than females (Rand, 1992). In contrast, no effects were seen on the final size or intensity of blue throat or ventral patches in striped plateau lizards *(Sceloporus virgatus)* when T implants were put in hatchling males and females (Abell, 1998).

Androgens affect aggressive behavior in both male and female *Sceloporus*. Moore (1986) found elevated T associated with territorial behavior during the nonbreeding season in male mountain spiny lizards *(Sceloporus jarrovi)*. This territorial aggression is affected by both castration and T replacement (Moore, 1987a,b; Moore and Marler, 1987). It is possible that aromatization is important for aggression in mountain spiny lizards, at least in females because aggressiveness in females is correlated with seasonal elevations in both T and estradiol (Woodley and Moore, 1999a), but ovariectomized T-implanted females lacked some elements of aggressive behavior observed in sham-operated controls (Woodley and Moore, 1999b). Elevated T and the associated territorial behavior have substantial energetic and survival costs in male *S. jarrovi* that appear to result from increases in energy expenditure without compensating increases in energy intake (Marler and Moore, 1989, 1991; Marler *et al.*, 1995). Comparable energetic and growth costs of experimentally elevated T levels are seen in male northern fence lizards *(Sceloporus undulatus hyacinthus)* (Klukowski *et al.*, 1998).

Sceloporus males can also show the short-term steroid hormone responses to encounters described for tree lizard and *U. stansburiana* males. Male *S. undulatus* show T elevations in response to a series of staged laboratory

encounters with other males during the breeding season, but not in response to similar staged encounters with females or other males outside the breeding season (Smith and John-Alder, 1999). Corticosterone levels in males are increased by both male and female encounters, whereas females do not show hormonal responses to either male or female encounters.

D. Sex Steroids and Behavior: Other Reptiles

Reptiles display a diversity of relationships between circulating steroid hormones and reproductive behavior. These relationships were comprehensively reviewed by Whittier and Tokarz (1992) for female reptiles and by Moore and Lindzey (1992) for male reptiles. Findings related to this topic also appear elsewhere in this chapter as they relate to TSD, parthenogenesis, and alternate reproductive phenotypes. We briefly review information presented in these earlier contributions by taxon and along with information that has been presented since these reviews were published.

As with other aspects of brain–behavior relationships in reptiles, steroid hormone effects on behavior are best understood in lizards and snakes. This is primarily due to the ease of husbandry and adaptability of many of these species to laboratory conditions. Many lizards are also very amenable to studies in the natural habitat. Exceptions to this focus on lizards and snakes are studies on sea turtles and tuataras. Both are of interest in part because of their endangered status. The hope is that better information on their reproductive biology may be applied to aiding in their conservation.

1. *Turtles*

This group has been the subject of a great deal of research related to TSD, but relatively little work has addressed hormone–behavior relationships in turtles. It is known that both luteinizing hormone (LH) and folliale-stimulating hormone (FSH) rise during the breeding period in female green sea turtles (*Chelonia mydas;* Licht *et al.,* 1979, 1980). P and T also rise during this period, although E_2 does not rise significantly. Patterns in the loggerhead sea turtle *(Caretta caretta)* show similarities—P, T, and corticosterone all decline over the course of the mating season through repeated nesting episodes (Wibbels *et al.,* 1990; Whittier *et al.,* 1997). Sea turtles are most easily sampled during the nesting period when they emerge onto beaches. Less information is available on steroid levels during other seasons. Rostal and coworkers (1998) approached this problem by sampling from captive Kemp's Ridley sea turtles *(Lepidochelys kempi)* under seminatural conditions. Male Kemp's Ridley turtles show T peaks several months prior to mating and these levels decline slightly by the mating season in March; they decline sharply following the cessation of breeding. Females exhibit peak levels of T, E_2, and P at the time of mating. Both T and E_2 decline sharply after mating, whereas P declines more slowly.

Social interactions also influence circulating steroid levels in green sea turtles during the mating period. Jessop and coworkers (1999a) found that female green sea turtles have higher levels of plasma corticosterone at nesting beaches (rookeries) with a high density of other nesting females than at comparable low-density nesting beaches. A combined measure of plasma androgens showed no difference with nesting-female density in this study. In contrast, male green sea turtles do show effects on plasma androgens related to social interactions (Jessop *et al.,* 1999b). Males near, or actually mounting, females have elevated androgen levels, whereas males that are the recipients of aggression from rival males or males that exhibit courtship damage resulting from this male–male aggression have lower circulating levels of androgen.

Although there are data on seasonal cycles in gonadal steroid hormones for other turtles and tortoises (e.g., Callard *et al.,* 1978; Lewis *et al.,* 1979; Sarkar *et al.,* 1996; Mahmoud and Licht, 1997; Schramm *et al.,* 1999; Shelby *et al.,* 2000), no studies have addressed the behavioral correlates of this variation. The single exception is work in musk turtles *(Sternotherus odoratus)* in which there is some evidence of both photoperiod and androgen control of sexual behavior (Mendonca, 1987a,b).

2. *Crocodilians, Tuatara, and Amphisbaenians*

The 21 species of crocodilians represent the most primitive extant members of the archosauromorph lineage that includes modern birds and the extinct dinosaurs. As with turtles, considerable information is available regarding steroid hormones and the process of TSD for crocodilians (represented by the American alligator, *Alligator missipiensis*). Some information is also

available regarding gametogenic cycles and circulating steroid hormones in the group (Guillette *et al.*, 1997). However, no experimental work has addressed the relationship of steroid hormones in crocodilians to behavior, and there are relatively few studies on the mating behavior of the group generally (e.g., Compton, 1981; Webb *et al.*, 1983; Thorbjarnason and Hernandez, 1993; Tucker *et al.*, 1998).

Data on tuataras, limited to a single extant species representing the order Sphenodontida, are similarly limited. Female tuatara exhibit a prolonged reproductive cycle, carrying eggs in the oviduct for 6–8 months and nesting only once every 4 years on average (Cree *et al.*, 1992). Tuataras appear to exhibit an associated reproductive pattern. Gametogenesis and testosterone levels in males follow an annual cycle—low during the winter, rising in the spring, and peaking in mid-summer to early autumn during the mating period. Female tuatara show elevated levels of E_2, and T during vitellogenesis, which fall at ovulation when P levels rise. Females also show elevations in plasma AVT during oviposition relative to during the nest digging and guarding stages that are probably associated with oviducal contractions (Guillette *et al.*, 1991).

As when this topic was last comprehensively reviewed (Whittier and Tokarz, 1992; Moore and Lindzey, 1992), no information is available regarding the relationship of steroid hormones to either reproduction or behavior in Amphisbaenians.

III. NEUROANATOMICAL SUBSTRATES OF SEXUAL BEHAVIOR IN REPTILES

Most work on the neural substrates of behavior in reptiles has focused on the squamate reptiles, lizards and snakes. This is the most speciose group and their typically small body sizes and ease of husbandry facilitates experimental studies in the laboratory as well as in the field. Research to date has focused primarily on the limbic system. The first studies established the role of limbic nuclei as critical integrative areas in the control of sexual behavior. Another area of research has been the metabolic and neurochemical differences between the sexes and within sexes across seasons and incubation conditions. The metabolic work has used 2-deoxyglucose (2DG) and cytochrome oxidase (CO) histochemistry. The neurochemistry work has focused on neurotransmitters, neuropeptides, and the expression and regulation of steroid receptor mRNAs.

A. Integrative Centers for Sexual Behaviors

The primary integrative centers for sexual behavior in reptiles are in the hypothalamus, as in other vertebrates (Sachs and Meisel, 1994; Pfaff *et al.*, 1994). The final common pathway for male-typical mounting and intromission behavior appears to be the preoptic area–anterior hypothalamus (POAH), whereas that of female-typical receptive behavior is the ventromedial portion of the hypothalamus (VMH; comparable to the ventromedial nucleus of the hypothalamus of rodents). Both of these areas are rich in steroid hormone receptors and their activity and neurochemistry responds to both steroid hormones and environmental signals. Experimental support for the critical importance of the POAH in regulating male-typical and VMH in regulating female-typical suites of reproductive behaviors comes mainly from two types of experiments.

Electrolytic lesion experiments involve creating localized damage in candidate regions followed by behavioral assays to assess disruptions of function. Lesions of the POAH impair courtship and copulatory behavior in male green anole lizards (*Anolis carolinensis;* Wheeler and Crews, 1978) and little striped whiptail lizards (*Cnemidophorus inornatus;* Kingston and Crews, 1994), whereas lesions of the VMH in the parthenogenetic whiptail lizard *C. uniparens* abolish receptive behavior; it is significant that only those lesions that encompassed the area containing ERs were effective (Kendrick *et al.*, 1995) (Fig. 12). Interestingly, POAH lesions also impair male-like pseudocopulatory behavior in the unisexual *C. uniparens*. This suggests that pseudosexual behavior in the descendant species of this pair is mediated by the same neural circuits responsible for copulatory and receptive behaviors in males and females of its ancestral species.

The second type of experiment providing critical support for the roles of the POAH and VMH in mediating sexual behaviors in reptiles involves intracranial implantation of minute amounts of steroid hormones directly into candidate brain regions of animals lacking gonads. This approach has been shown to effectively restore male-typical sexual behaviors in rats

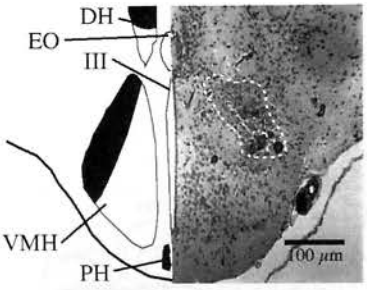

FIGURE 12 Composite illustration of the ventral portion of the hypothalamus of *Cnemidophorus uniparens* at the level of the ventromedial hypothalamus (VMH). Left: The outlines of the VMH, dorsal hypothalamus (DH), the ependymal organ (EO), the third ventricle (III), and the periventricular hypothalamus (PH). The black regions represent the location of estrogen receptor mRNA. Right: Photomicrograph of brain tissue stained with cresyl violet. The dashed white line demarks the tissue damage caused by an electrolytic lesion. Only those lesions of the dorsolateral VMH effectively prevented estrogen induction of sexual receptivity. From Kendrick *et al.* (1995).

and other mammals (reviewed in Sachs and Meisel, 1994). The implantation of androgens directly into the POAH reinstates courtship and copulatory behaviors in castrated male green anoles (Morgentaler and Crews, 1978). Likewise, intracranial implantation of androgen into the POAH induces copulatory behaviors in both castrated male whiptails (*C. inornatus;* Rozendaal and Crews, 1989) and the parthenogenetic *C. uniparens* (Mayo and Crews, 1987) (Fig. 13). Intracranial implantation of P rather than androgen is also effective in restoring courtship and copulatory behavior in a subset of *C. inornatus* males that are sensitive to intraperitoneal P implants (Crews *et al.*, 1996b).

As with male-typical sexual behavior, the implantation of estrogen directly into the VMH of ovariectomized female and unisexual whiptail lizards reinstates receptive behavior (Wade and Crews, 1991) (Fig. 13). This finding agrees well with the characterized distribution and regulation of both ERs and PRs in this brain region (see later).

B. Variation in Brain Nuclei and Neuron Soma Sizes

Several sex differences in reptilian brain nuclei or soma sizes are best characterized in the whiptail lizards and green anoles. In the sexual species of whiptail lizard, *C. inornatus,* males have larger POAH than do females, whereas females have a larger VMH (Crews *et al.,* 1990). These sexual dimorphisms in size are under the control of gonadal androgens in the male (Fig. 14). That is, castration of breeding animals results in a reduction in the area of the preoptic area (POA) and an enlargement in the area of the VMH, whereas androgen replacement therapy reverses these effects of castration. It is significant that only the male shows these responses to hormonal manipulation. These overall differences in nucleus size are correlated with differences in soma size in these areas. Male *C. inornatus* have larger soma sizes in the POA, whereas females have larger soma sizes in the VMH (Wade and Crews, 1992). Interestingly, the brains of the all-female *C. uniparens* show patterns similar to those of females of the sexual species, despite the fact that these females regularly show male-like pseudosexual behavior. This finding is also true when the parthenogenetic females are sex-reversed using fadrozole, an inhibitor of aromatase that effectively induces male development in *C. uniparens* (Wennstrom and Crews, 1995; Wennstrom *et al.,* 1999). This result indicates that, although useful, measurements of brain nucleus volume and soma size probably do not reflect many important differences in function. We return to this topic later when considering metabolic capacity, neurotransmitter function, and regulation of steroid-hormone-receptor expression.

Seasonal variation in the size of brain areas has been documented in a variety of vertebrate species, particularly in the song system of many birds (see Ball and Balthazart, Chap. 32 in this volume; Schlinger and Brenowitz, Chap. 33 in this volume). Many reptiles also show seasonal reproduction, but the neural correlates of this seasonality are less well documented. In the Canadian red-sided garter snake (*Thamnophis sirtalis parietalis*) the volume of the POA varies seasonally in females, but not in males (Crews *et al.,* 1993). The POA of female snakes is smaller than that of males during the hibernation period. The lack of variation in males may be related to the fact that, unlike the songbirds that are the focus of most studies, these garter snakes exhibit a dissociated reproductive pattern in which seasonal mating behavior and gonadal steroid hormone peaks are temporally offset (Crews, 1991).

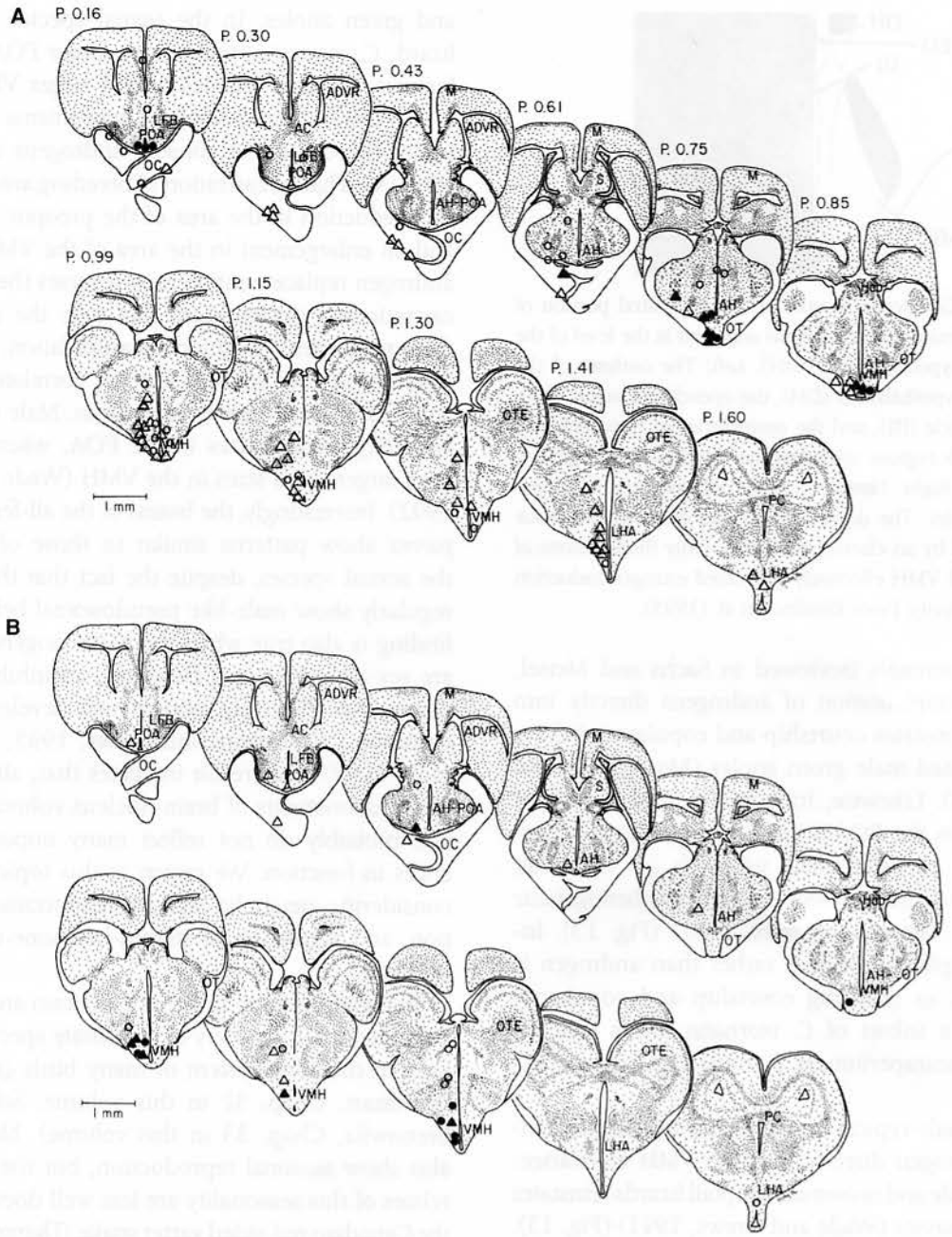

FIGURE 13 Frontal sections through the brain of a representative whiptail lizard, showing locations of the approximate center of hormone implants that elicited (A) mounting and copulatory behavior or (B) sexual receptivity in whiptail lizards. Numerals indicate distance posterior to zero point. (A) Solid triangles indicate locations of dihydrotestosterone implants that resulted in male-like pseudocopulatory behavior in ovariectomized parthenogenetic whiptails *(Cnemidophorus uniparens)*, whereas solid circles indicate implants that result in male-typical copulatory behavior in castrated male whiptails *(C. inornatus)*. Open symbols represent placement of implants that failed to respond. (B) Solid triangles indicate locations of estrogen implants that resulted in female-like sexual receptivity in ovariectomized parthenogenetic whiptails *(Cnemidophorus uniparens)*, whereas solid circles indicate implants that result in female-typical sexual receptivity in ovariectomized female whiptails *(C. inornatus)*. Open symbols represent placement of implants that failed respond. AC, anterior commissure; ADVR, anterodorsal ventricular ridge; AH, anterior hypothalamus; LFB, lateral forebrain bundle; LHA, lateral hypothalamic area; OC, optic chiasm; OT, optic tract; OTE, optic tectum; PC, posterior commissure; POA, preoptic area; VMH, ventromedial hypothalamus.

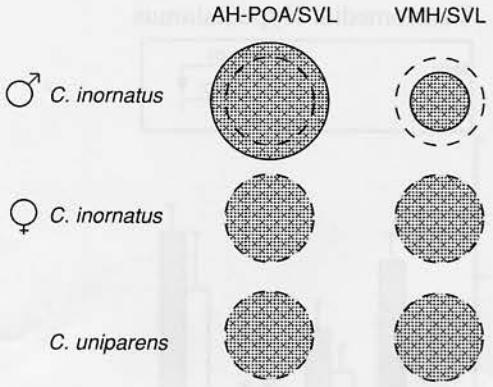

FIGURE 14 Schematic representations of the volumes of the sexually dimorphic areas in the brain relative to body size in sexual and parthenogenetic whiptails. To aid in comparison, the volume of the POAH and the VMH of female *C. inornatus* is represented as a solid outline in other figures to indicate significant differences (SVL refers to snout-vent length, a standard measure of body size in reptiles).

Information has become available on sexual dimorphisms in both the brain stem and limbic system of green anoles. Sexual and aggressive behaviors in green anoles are well characterized (Crews, 1975b; Crews *et al.*, 1978; Andrews and Summers, 1996; Propper *et al.*, 1991). The control of these behaviors by gonadal steroid hormones in green anoles has also received a good deal of attention (Valenstein and Crews, 1977; Crews *et al.*, 1978; Crews and Morgentaler, 1979; Tokarz and Crews, 1979, 1980, 1981; Jones *et al.*, 1983). Males perform push ups, as in many male lizards, and are also able to greatly extend a red-pigmented portion of skin on the ventral side of the neck (termed the dewlap) through flexion of the hyoid apparatus (Crews, 1975a); although female green anoles have similar pigmentation in the gular region and use this as an aggressive signal, neither the skin nor the hyoid is as well developed as in males (Crews, 1975b). Both of these signals are used in social contexts, with dewlap extension being shown only in males. The muscle primarily responsible for dewlap extension, the ceratohyoideus muscle, is innervated from the nucleus ambiguus (AmbX) as well as the glossopharyngeal portion of the AmbX and the ventral portion of the motor nucleus of the facial nerve (AmbIX/VIImv). Neurons in both brain regions are larger in males than in females (Wade 1998). The motor neuron number does not vary by sex or across the breeding and nonbreeding seasons, but nerve cross-sectional area and both muscle fiber size and number are greater in males than females (O'Bryant and Wade, 2000a). However, there is no consistent relationship between either the breeding and nonbreeding season or androgen treatment (T propionate) and these characteristics. Another study found that, unlike some other vertebrate systems in which the sexes differ in their display behavior (Gurney, 1981; Devoogd and Nottebohm, 1981; Kelley *et al.*, 1988; Bass and Baker, 1990), green anoles show no sex difference in the dendritic arborization of motoneurons in AmbX or AmbIX/VIImv (O'Bryant and Wade, 2000b). These findings suggest that changes in this system during adulthood do not underlie sex or seasonal differences in the dewlap-extension behavior. O'Bryant and Wade propose that this may be due to the fact that, although the extension of the dewlap is associated with male courtship, females also lower the hyoid, thereby exposing the patch of red.

The described research with whiptail and green anole lizards focused on species with the familiar pattern of GSD. The other end of the genotype–environment spectrum is represented by species that exhibit TSD. In the leopard gecko *(Eublepharis macularius),* both low and high incubation temperatures produce females, whereas intermediate temperatures result in male determination and differentiation (Fig. 4). Interestingly, sexuality covaries with incubation temperature somewhat independently of gonadal sex, such that females produced at higher temperatures are masculinized compared to females incubated at low temperatures. This variation is reminiscent of the intrauterine position effect in mammals and may provide a powerful experimental system for addressing the causes and consequences of prenatal hormonal effects as well as maternal effects on offspring phenotypes.

Coomber, Crews, and Gonzalez-Lima (1997) found that females from male-biased incubation temperatures had larger POA volumes than those from female-biased incubation temperatures (Fig. 15). There are parallel differences in the VMH, but this varies with age; for example, old females have a larger POA and VMH than do young females. Differences are also seen in males with age, but these contrast with those found in females (Crews *et al.*, 1997). Young males show larger volumes for the POA and VMH than do older males. Sociosexual experience does not have strong effects on brain nucleus

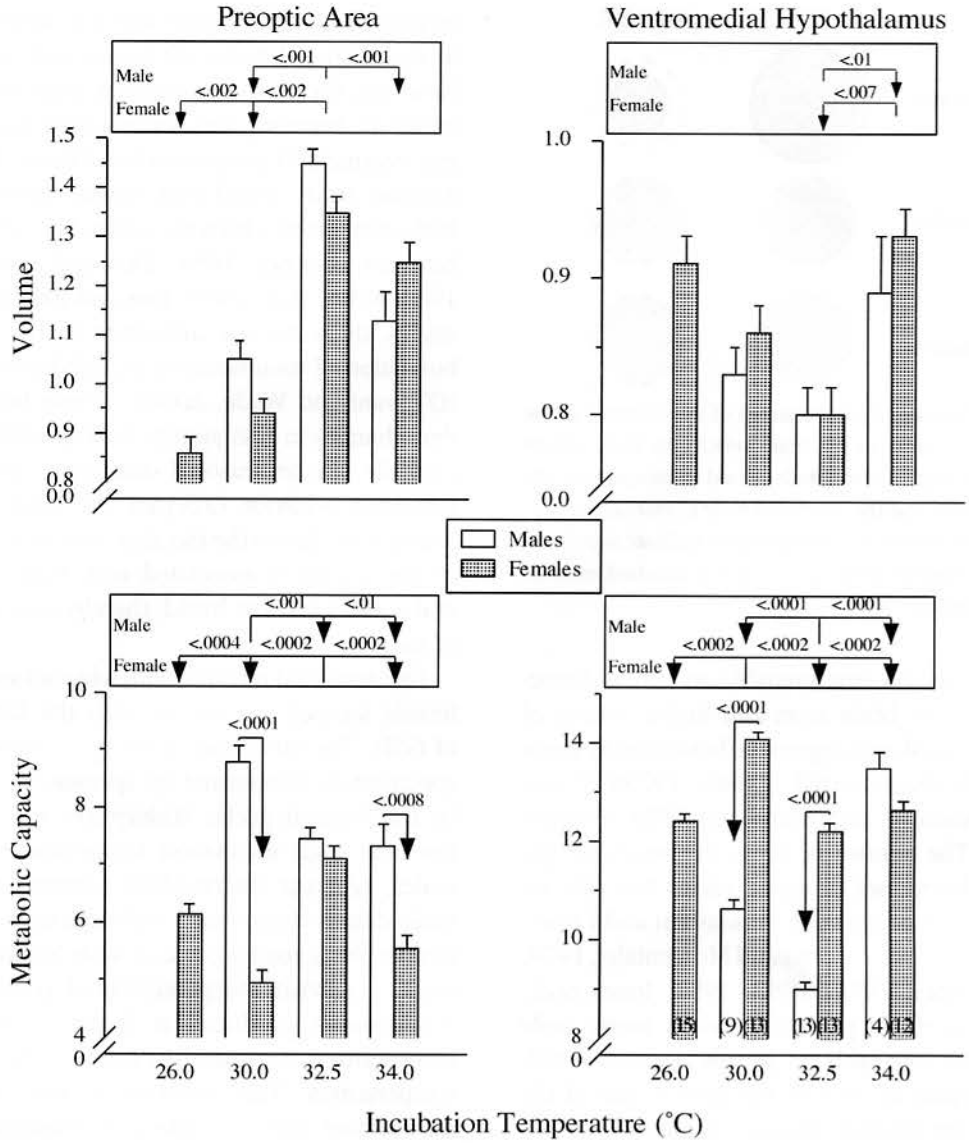

FIGURE 15 Effect of incubation temperature and gonadal sex on the volume (top panels) and cytochrome oxidase (CO) activity (μmol/min/g tissue wet weight) (bottom panels) of the preoptic area (POA) (left panels) and ventromedial hypothalamus (VMH) (right panels) in the leopard gecko (*Eublepharis macularius*). Volumes are normalized by entire forebrain volume. Significant differences (entries are p values) within each sex are illustrated in boxes above each panel, indicating the effect of incubation temperature. Significant differences between the sexes are illustrated above bars, indicating the effect of gonadal sex. Sample sizes are in parentheses. Means are depicted with vertical bars representing standard error. Data from Coomber *et al.* (1997).

volume (possibly an increase in POA volume in female geckos from female-determining temperatures), but experience does strongly affect the metabolic capacity of brain areas functionally linked to sexual and aggressive behavior (see later).

IV. METABOLIC INDICATORS OF NEURAL ACTIVITY

Although informative, measurements of brain nucleus volume and soma size represent only an indirect

assessment of function. One exciting development in our ability to assess sex differences in the neural substrates of sexual behavior has been the introduction and implementation of measurements of acute metabolic activity and sustained metabolic capacity in the brains of reptiles and other vertebrates. The 2-DG technique utilizes the dependence of neurons on glucose as a source of energy and an analog of glucose that can be taken up by cells, but not metabolized. This allows ^{14}C-labeled 2-DG to accumulate in cells and this accumulation can be assessed by the relative amounts of radioactivity present in tissue sections (Cada *et al.*, 1995). CO catalyzes the rate-limiting step in oxidative respiration in brain tissue and levels of this enzyme provide a useful indicator of the total metabolic capacity (Wong-Riley, 1989).

A. Acute Metabolic Activity Associated with Behavioral State: 2-Deoxyglucose Utilization

Even when no morphometric differences are apparent across behavioral phenotypes, indicators of metabolic activity can demonstrate differences in function. Rand and Crews (1994) found that the acute metabolic activity of the parthenogenetic *C. uniparens* depended on whether they were displaying male-like or female-like pseudosexual behavior (Table 2). Specifically, animals displaying male-like pseudocopulatory behavior showed a sixfold greater accumulation of 2-fluoro-2-DG in the medial POA than did animals showing female-like behavior. Conversely, individuals showing female-like receptive pseudosexual behavior exhibited a greater accumulation of 2-DG in the VMH.

TABLE 2
Optical Density Scores of the Medial Preoptic Area and Ventromedial Hypothalamus in *Cnemidophorus uniparens*

Behavior	(*N*)	*mPOA score*		*VMH score*
Courtship	(6)	-5.86 ± 0.79	+	$+4.58 \pm 1.03$
		+		*
Receptive	(5)	$+0.12 \pm 1.70$		$+0.66 \pm 0.63$

[a] Subjects exhibit either male-like pseudocopulatory (courtship) or female-like (receptive) behavior. Lower scores indicate higher 2DG accumulations; + indicates significance at $P = 0.008$; * indicates significance at $P = 0.006$.

The results from *C. uniparens* are paralleled across seasons in red-sided garter snakes. Male red-sided garter snakes that actively court females show a significantly higher 2-DG accumulation in the POAH than males who either are exposed to females but fail to court or males that are not exposed to females (Allen and Crews, 1992). Interestingly, simply being exposed to females increases the overall 2-DG accumulation in garter snake males. These results suggest both a generalized arousal effect as well as a more specific effect of active courting that is restricted to the brain region very directly involved in mediating this behavior.

Radiolabeled 2-DG has also been used to study patterns of neural activity associated with the change from a receptive to an unreceptive state in female red-sided garter snakes (Mendonça and Crews, 2001). On emergence from hibernation, females initially are receptive to male courtship behavior but become unreceptive immediately following mating. Females that are courted and then mate have significantly higher activity in the POA and significantly lower activity in the VMH compared to females who are courted but do not mate (Fig. 16). Because intromission during mating is responsible for the loss of sexual receptivity in the female (Mendonça and Crews, 1990, 2001; Ross and Crews, 1977; Whittier and Crews, 1989; Whittier *et al.*, 1985, 1987), the injection of a local anesthetic (tetracaine or lidocaine) into the cloacal region desensitizes the female to mating stimuli (Mendonça and Crews, 1990, 2001). Not only does this treatment prevent the mating-induced surge in estrogen levels in the plasma and subsequent ovarian recrudescence, but the pattern of 2-DG accumulation in tetracaine-treated females is similar to courted but unmated females and to females exposed only to other females. These results suggest that in the female red-sided garter snake sensory input from the cloaca during mating alters patterns of metabolism in those brain areas most often associated with sexual receptivity. The increased activity in the POA accompanied by a decrease in activity in the VMH after mating supports the hypothesis that mating initiates a neuroendocrine reflex that results in a loss of receptivity in female red-sided garter snakes.

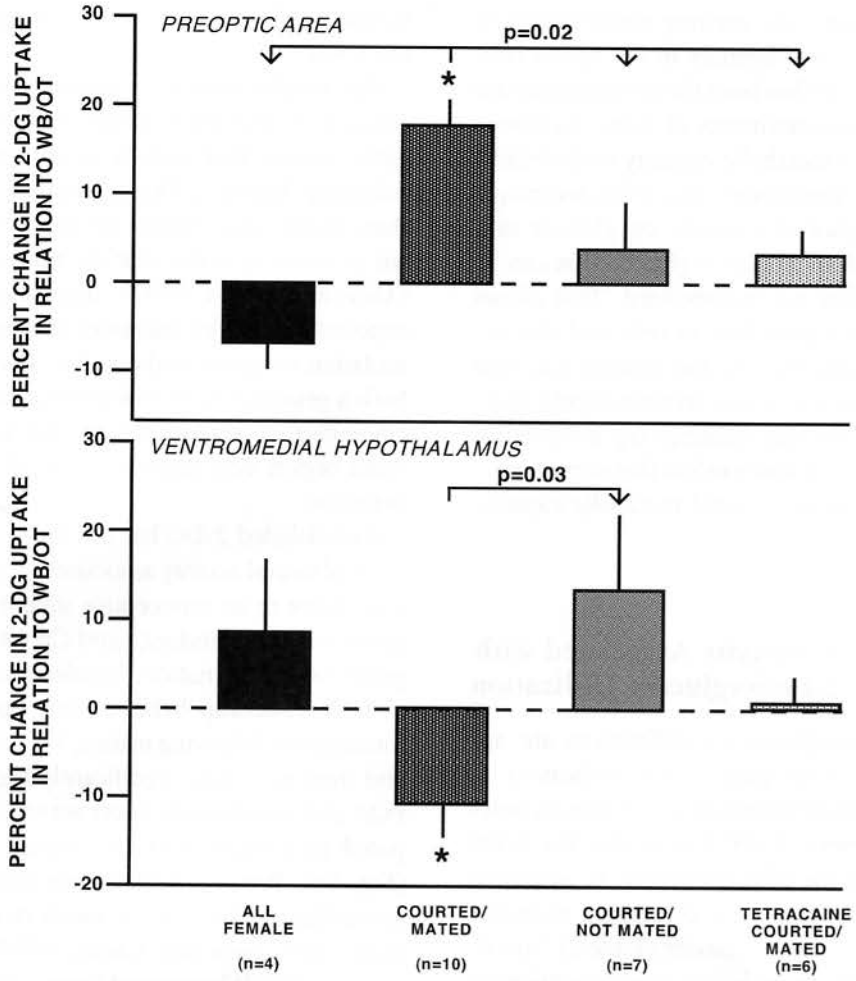

FIGURE 16 Mean relative change in 2-deoxyglucose (2-DG) uptake in the red-sided garter snake *(Thamnophis sirtalis parietalis)*. Values for different treatment groups relative to whole brain/optic tract (WB/OT). The dashed line indicates background brain levels (e.g., zero difference from background). A positive change represents higher accumulation; a negative change represents lower accumulation than background. The vertical lines represent 1 standard error. The asterisk represents significant differences. The p level of the difference is indicated. A. Uptake of 2-DG in the preoptic area–optic tract. B. Uptake of 2-DG in the ventromedial hypothalamus–optic tract.

B. Metabolic Capacity Associated with Behavioral Phenotype: Cytochrome Oxidase Histochemistry

As with brain nucleus volume, a variety of factors influence metabolic capacity in the brain of leopard geckos. These include incubation temperature, age, and sexual experience. Incubation temperature affects CO activity in both females and males, although the effect varies depending on the brain nucleus being considered. Females from a male-biased incubation temperature have increased metabolic capacity of the anterior hypothalamus (AH), external amygdala, dorsolateral hypothalamus, dorsoventricular ridge, nucleus sphericus, lateral septum, and striatum, but do not increase capacity in the posterior hypothalamus or periventricular POA (Fig. 17). Of particular interest is the finding that young females from a male-biased incubation temperature show greater CO levels in the medial POA than young females from a female-biased

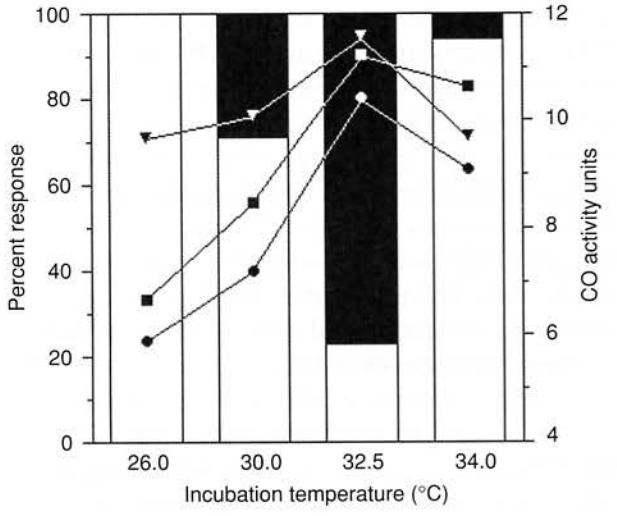

FIGURE 17 Relationship between sex ratio, aggressive behavior, and cytochrome oxidase activity in the amygdala in female leopard geckos. Sex in the leopard gecko is determined by the incubation temperature of the egg; the sex ratio produced at the temperatures indicated is reflected in the bar graph (proportion male is indicated by darkened areas of bars). The proportion of females responding aggressively toward a courting male indicated by squares. Cytochrome oxidase activity in the nucleus sphericus and external amygdala of females from these same incubation temperatures are shown by inverted triangles and circles, respectively. In reptiles, the nucleus sphericus and external amygdala are homologous to the medial and basolateral amygdala of mammals, respectively; as in mammals, both areas are involved in the control of aggression. Thus, embryonic experience with temperature affects the level of aggressive behavior and brain metabolism in the amygdala of adult females.

incubation temperature. Females from a male-biased incubation temperature are more aggressive and less sexually attractive than females from a female-biased incubation temperature. CO levels in male leopard geckos are also influenced by incubation temperature, with males from a female-biased incubation temperature having greater levels of CO in the POA and VMH than males from a male-biased incubation temperature. Such findings are reminiscent of the finding that in mice males positioned between two females *in utero* are more sexually active than their male siblings positioned between two males (cf. Clark and Galef, 1995).

Age and sexual experience are also important factors determining metabolic activity in limbic nuclei in both male and female geckos. For example, age is associated with a decrease in the size of the POA and VMH in males, but not in females. CO activity increases in the POA, nucleus sphericus, and external amygdala (reptilian counterparts to the mammalian amygdala) with age in males, but the precise effects of age on CO levels in different brain nuclei in female leopard geckos vary with incubation temperature, indicating a complex interaction of these factors (Coomber *et al.*, 1997).

Sexual experience also influences the metabolic capacity of limbic nuclei in leopard geckos, again in complex ways (Crews *et al.*, 1997). Several nuclei, including the VMH and AH, have higher metabolic capacities in sexually experienced males than in sexually inexperienced males. In contrast, there is no difference with experience in the POA or several other limbic nuclei in males. On the other hand, sexually experienced female leopard geckos show a higher CO abundance in the POA than sexually inexperienced females regardless of incubation temperature history; the results were mixed for other nuclei.

Sakata and coworkers (2000) assessed functional connectivity among limbic nuclei in the leopard gecko by analyzing covariance patterns in metabolic capacity, as revealed by quantitative CO histochemistry. As previously indicated, incubation temperature during embryonic development influences an individual's aggressive and sexual behaviors in adulthood. For example, an increase in incubation temperature results in an increase in adult aggressivity in both males and females. Correlated with this are increased amounts of CO in the AH and both the septum and POA. Similarly, female-typical sexual behaviors decline with increasing incubation temperature, and the correlations between the VMH and both the dorsoventricular ridge and septum were significant only in females. Correlations among preoptic, hypothalamic, and amygdalar areas tend to be distributed across both sexes, suggesting that there may exist shared pathways underlying the expression of male-typical and female-typical behaviors.

Thus, a variety of factors including gonadal sex, age, sexual experience, and incubation temperature history influence the volume and metabolic capacity of brain nuclei and the connectivity among these nuclei in leopard geckos. The dominant influence, however, is incubation temperature. This work provided the first unequivocal demonstration that factors other

than gonadal sex and gonadal hormones can influence the sexual differentiation of the brain in vertebrates.

V. NEUROCHEMICAL BASES OF SEXUAL AND AGGRESSIVE BEHAVIOR IN REPTILES

The relationship of neurotransmitters and neuropeptides to sexual and other behaviors in reptiles has received relatively little attention. Propper and coworkers (1992a) examined the distribution of AVT in the green anole and found labeling in the cortex, around the olfactory ventricle, in the diagonal band of Broca, and in the amygdala area, dorsal ventricular ridge, striatum, nucleus accumbens, septum, VMH, lateral hypothalamus, medial forebrain bundle, median eminence, pars nervosa, nucleus of the solitary tract, locus coeruleus, cerebellar cortex (granular layer), dorsal part of the nucleus of the lateral lemniscus, substantia nigra, and myelencephalon. There is generally greater intensity of staining in males than females. The distribution of AVT immunoreactivity has also been examined in a turtle *(Pseudemys scripta)* and a python *(Python regius)* (Smeets *et al.*, 1990). No sex differences were described in vasopressin-like or oxytocin-like (presumably AVT and mesotocin) immunoreactivity in the brain of the chameleon, although differences were found for females across the ovarian cycle (Bennis *et al.*, 1995). This variation in AVT also occurs in female green anoles, in which females with large preovulatory follicles have higher AVT concentrations in the supraoptic area than do females with small preovulatory follicles (Propper *et al.*, 1992b).

Dominance interactions influence monoamine metabolism in male lizards. For example, aggressive interactions increase plasma epinephrine and norepinephrine in male green anoles and these levels are higher in males winning encounters than in males that lose (Summers and Greenberg, 1994). This response and the speed of the correlated eyespot darkening are reduced by castration, suggesting an influence of testosterone. Both dominant and subordinate male anoles show changes in central monoamine metabolism following aggressive encounters, but these changes are more pronounced in subordinates. Subordinate male green anoles show elevated ratios of 5-hydroxyindoleacetic acid (5-HIAA) to 5-hydroxytryptamine (5-HT) and the substrate for 5-HT, 5-hydroxytryptophan (5-HTP), indicating enhancement of both serotonin turnover and production, 1 hour after an encounter with a dominant individual (Summers and Greenberg, 1995). The difference between subordinate and both dominant and control males diminishes thereafter. Dominant males show broadly similar patterns, but return to baseline turnover levels more rapidly. Neither the dopaminergic nor adrenergic systems showed similar patterns, indicating this response is specific to the serotonergic system. This serotonergic response is also regionally specific. The nucleus accumbens and hippocampal cortex show the most dramatic changes 1 hour following an aggressive interaction, but the medial and lateral amygdala show a more delayed response with serotonergic activity, peaking at 1 week postinteraction in subordinate males (Summers *et al.*, 1998). The amygdalar region is important in regulating sexual and aggressive behaviors in green anoles (Greenberg *et al.*, 1984).

Both plasma and brain-region-specific alterations in monoamine metabolism can also be induced in male anoles by manipulating a key aggressive signal and exposing males to a mirror (Korzan *et al.*, 2000a,b). Masking a male's eyespot with green paint suggests a less aggressive or subordinate opponent when the animal observes this opponent (itself) in a mirror. Males whose eyespots were painted green showed the highest frequency of biting behavior. These males also showed elevated plasma levels of dopamine, epinephrine, and norepinephrine compared to isolated controls and males whose eyespots were painted black. In the brain, males with green-painted eyespots showed increased serotonergic and adrenergic activity (but lower dopaminergic activity) in the subiculum (dorsal cortex), hippocampus, nucleus accumbens, and medial amygdala compared to males whose eyespots were darkened (suggesting an aggressive or dominant opponent) (Fig. 18).

The patterns of monoamine metabolism in mountain spiny lizards *(Sceloporus jarrovi)* are similar to those in anoles—higher serotonergic activity is seen in non-territorial satellite (subordinate) males than in territorial males (Matter *et al.*, 1998). As with anoles, aggressive defense of territory in *S. jarrovi* males results in increases both in 5-HTP and in 5-HIAA/5-HT ratios. These interactions also increase the activity of the

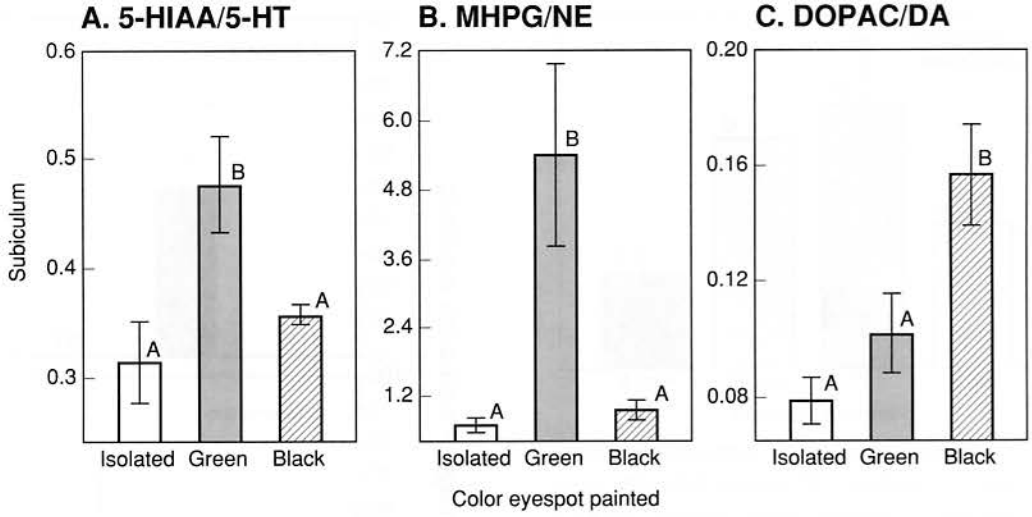

FIGURE 18 Monoaminergic profiles in the dorsal cortex (subiculum) of male green anoles following 10 minutes exposure to mirror presentations in one of two conditions (gray bars, subject male's eyespot was painted green; black bars, subject's eyespot was painted black; white bars, isolated controls). Neurotransmitter activity is depicted as ratios of important catabolite levels to levels of the neurotransmitter itself for (a) serotonin (5-HIAA/5-HT), (b) norepinephrine (MHPG/NE), and (c) dopamine (DOPAC/DA). Bars are mean ± S.E.M.; means with a different letter labels are significantly different. 5-HIAA, 5-hyddroxyindoleacetic acid; 5-HT, 5-hydroxytryptophan; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; MHPG, 4-hydroxy-3-methoxyphenylglycol; NE, norepinephrine. Reprinted from Korzan *et al.* (2000a) with permission from Elsevier Science.

central dopamine and epinephrine systems. Plasma levels of norepinephrine and epinephrine also rise rapidly during restraint stress or following territorial interactions in *S. jarrovi* (Matt *et al.*, 1997).

The rapid alterations in serotonergic metabolism during aggressive interactions in male green anoles may be modulated by alterations in circulating steroid hormones. In green anoles, serotonin turnover (5-HIAA/5-HT) was enhanced in the hippocampus and medial amygdala 20 minutes after males received low-dose systemic injections of corticosterone (1.6–2.0 mg/kg), but not following 10-fold-higher corticosterone doses (16–20 mg/kg) (Summers *et al.*, 2000) (Fig. 19). Testosterone injections (1.6–2.0 mg/kg) enhanced serotonin turnover in the hippocampus, but not the medial amygdala. There were no changes found in several other brain regions or in the activity of other monoaminergic systems. The authors note the possibility that the injected testosterone could have been converted before having this effect. The brain of green anoles does show both aromatase and 5α-reductase activity (Wade, 1997) and at least aromatase activity is important in some behavioral contexts (Winkler and Wade, 1998). Aggressive interactions influence plasma steroid levels in green anoles and other male lizards (Greenberg and Crews, 1990; Knapp and Moore, 1995, 1996). Testosterone levels are also typically elevated during the breeding season and influence aggression (Moore and Crews, 1986; Moore, 1986, 1988), although plasma T levels do not necessarily change following an aggressive encounter (e.g., Moore, 1987b). The steroid hormone mediation of serotonergic metabolism demonstrated in green anole males was suggested as part of a mechanism enabling an individual to respond to changing social situations.

Differences are also found with social status in female anoles (Summers *et al.*, 1997). Females housed with males singly did not differ from isolated females. However, there were differences among females housed in groups of five with a male. In contrast to results obtained with green anole males, dominant females in this experiment showed higher 5-HT and dopamine (DA) activity in the telencephalon than did subordinate females, whereas subordinate females showed higher serotonergic activity in the brain stem. It was suggested that the heightened serotonergic activity in dominant

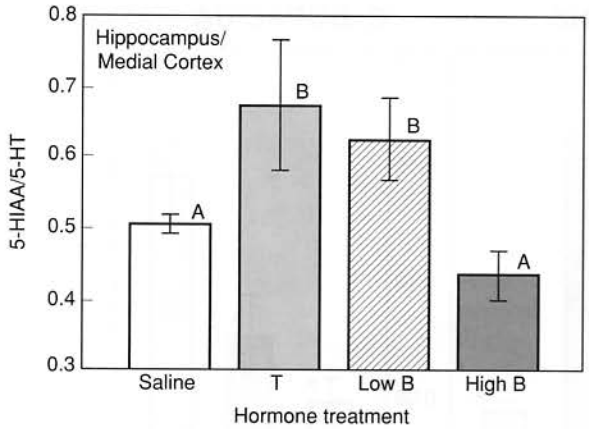

FIGURE 19 Serotonergic activity in the hippocampus and medial cortex of male green anoles 20 minutes following an intraperitoneal injection of a saline control solution, 10 μg T, 10 μg corticosterone (low B), or 100 μg corticosterone (high B). Serotonergic activity is assessed as the ratio of the primary metabolite 5-hydroxyindoleacetic acid (5-HIAA) to serotonin (5-HT). Bars are mean ± S.E.M.; Means with a different letter label are significantly different. Redrawn from Summers *et al.* (2000).

females is more directly related to interactions with males than to those with other females.

DA also is integral to the display of copulatory behaviors in male mammals and birds. Woolley and colleagues (2001) determined that a DA D1 receptor agonist facilitates the display of courtship and copulatory behaviors in both castrated sexual *(C. inornatus)* and ovariectomized parthenogenetic *(C. uniparens)* whiptail lizards. In both species, the D1 agonist SKF 81297 increases the proportion of individuals mounting and decreases the latency to mount (Fig. 20). Moreover, there is a difference in sensitivity to the agonist between the species—mounting is elicited at a lower dose in *C. uniparens* than in *C. inornatus*. This suggests that, as is the case for sensitivity to exogenous estrogen (see previous discussion), the heightened sensitivity in the triploid parthenogen is due to the increased ploidy, indicating that the parthenogen may have elevated levels of D1 receptor in limbic brain areas that modulate courtship behavior. Not only does this work extend to reptiles the central role of DA in the modulation of copulatory behavior, it also indicates that DA can elicit male-typical mounting behavior from both a "male" *(C. inornatus)* and a "female" *(C. uniparens)* brain.

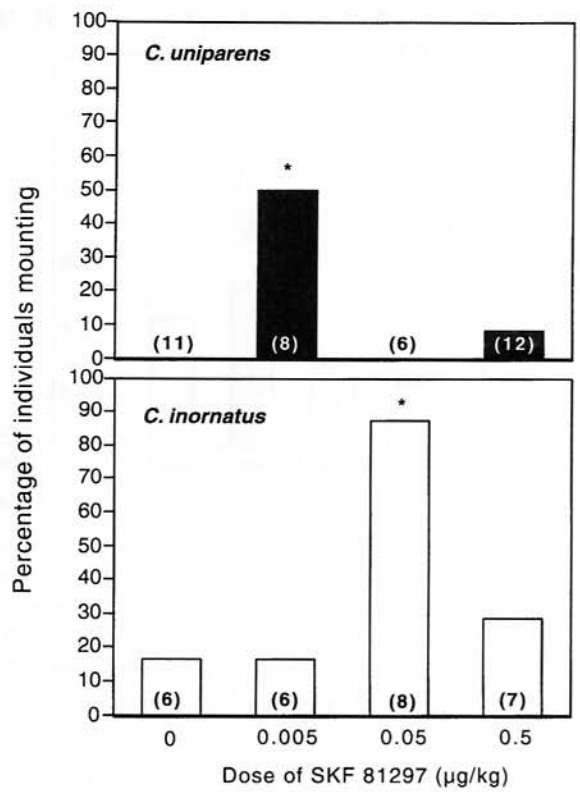

FIGURE 20 The percentage of individuals of each species mounting across all treatment groups (in *Cnemidophorus uniparens* (black bars) and *C. inornatus*(white bars)). In each species, an asterisk indicates a dose that is significantly different than vehicle treatment ($P < 0.05$). Redrawn from Woolley *et al.* (2001).

VI. DISTRIBUTION AND REGULATION OF SEX STEROID HORMONE RECEPTORS

Studies of sex steroid receptors in the brain of reptiles have focused exclusively on lizards and snakes. These studies have taken one of three approaches. Early studies examined the accumulation of radiolabeled sex steroid hormones T, DHT, or E_2 in specific brain nuclei. Later, steroid hormone receptors have been cloned from reptiles, allowing cellular-level localization of their mRNAs and studies of the factors influencing their abundance. Immunocytochemistry has also been used to map androgen receptor distribution.

A. Distribution

The accumulation of tritium-labeled E_2, T, and DHT is found at a variety of sites in the brain of green anole

lizards (Morrell *et al.*, 1979). Halpern and coworkers (1982) performed a similar study with labeled E_2 and T in the brain of red-sided garter snakes. These studies, similar to those in mammals and other vertebrates (see Morrell and Pfaff, 1978), reveal substantial E_2 binding in the POA, AH, amygdaloid nuclei, VMH, and posterior hypothalamic nuclei, with lighter labeling in several areas including the septum, torus semicircularis, central gray, and some brain-stem areas. In the green anole, the binding of tritiated T and DHT is very similar to patterns for tritiated E_2, but there are some differences in the pallial and the mesencephalic tegmental area.

A series of studies have explored the location and hormonal and social regulation of the mRNAs encoding the estrogen receptor-α (ERα), androgen receptor (AR), and progesterone receptor (PR) in whiptail lizards. These studies have elucidated the brain regions where each of these receptor mRNAs is expressed, variation in receptor mRNA abundances over the course of the ovarian cycle, species differences in expression levels and behavioral correlates of this expression, sex and species differences in the hormonal regulation of this expression, developmental influences on the sexual differentiation of hormonal responsiveness in receptor mRNA expression, and documented social influences on ER and PR mRNA abundance in the hypothalamic nuclei.

The mapping of the distribution of steroid receptor mRNA expression has relied on cloning portions of genes coding for sex steroid hormone receptors using reverse transcription–polymerase chain reactions (Young *et al.*, 1994). The resulting clones were used to generate riboprobes for use in *in situ* hybridization. The partial ER clone generated is homologous to ERα, although an isoform of the ER lacking exon 4 has been identified in whiptail lizards; a homolog of this ER isoform has also been found in rats (Skipper *et al.*, 1993). In general, *in situ* hybridization studies have documented the expression of ERs and ARs in the same regions as previous work using tritiated label to identify neurons concentrating sex steroid hormones, although some additional regions not found using steroid autoradiography have been described (see later).

Strong ER mRNA expression is found in the periventricular and medial POA, AH, periventricular nuclei of the hypothalamus, septal nuclei, the optic tectum, and the dorso-, postero-, and ventromedial hypothalamus

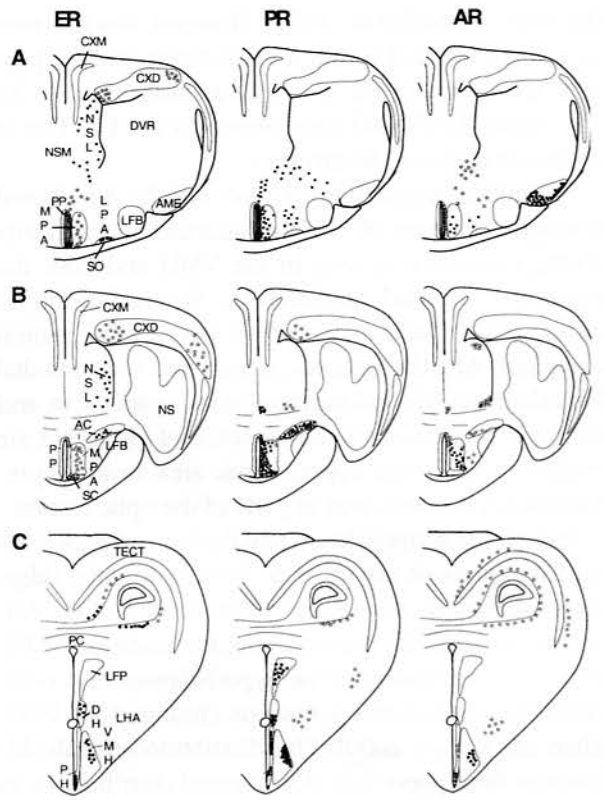

FIGURE 21 Distribution of cells expressing steroid receptor mRNA in selected sections of the brain of whiptail lizards. Shown are the positions of cells expressing mRNA for estrogen receptor (ER column), progesterone receptor (PR column), and androgen receptor (AR column) in the right half of brain sections. Solid circles indicate heavily labeled cells and open circles indicate lightly labeled cells. AC, anterior commissure; AME, nucleus externus amygdalae; CXD, cortex dorsalis; CXM, cortex medialis; DH, nucleus dorsalis hypothalami; DVR, dorsoventricular ridge; LFB, lateral forebrain bundle; LHA, lateral hypothalamic area; LPA, lateral preoptic area; LTP, lentiformis thalami pars plicta; MPA, medial preoptic area; NS, nucleus sphericus; NSL, nucleus septalis lateralis; NSM, nucleus septalis medialis; PC, posterior commissure; PH, nucleus periventricularis hypothalami; PP, nucleus periventricularis preopticus; SC, nucleus suprachiasmaticus; SO, nucleus supraopticus; TECT, optic tectum; VMH, nucleus ventromedialis hypothalami. Redrawn from Young *et al.*, *J. Comp. Neurol.* **347**, 288–300, copyright © 1994, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

(Fig. 21). Weaker labeling is found in the dorsal cortex, near the nucleus accumbens, the lateral and medial septal areas, and the supraoptic nucleus. Previous work with green anole lizards suggested little difference in androgen- and estrogen-concentrating neurons in

the brain (Morrell *et al.*, 1979). However, this previous study used tritiated E_2 and T, respectively, and it is possible that some of the T label was aromatized to E_2 (see Wade, 1997) and then bound to the ER. This is discussed at greater length later.

PR mRNA expression is also widely distributed through the brain of whiptail lizards. An especially strong expression is seen in the VMH and both the periventricular and medial POA. Strong labeling is also found in the medial septum, lateral POA, central amygdala, AH, the postero-, dorso- and ventromedial hypothalamus, the lentiformis thalamis pars plicta, and the torus semicircularis. Lower levels of PR mRNA are evident in the lateral hypothalamic area, near the premammilary nucleus, and in parts of the optic tectum.

In both the whiptail lizard and the leopard gecko, AR mRNA expression occurs in the dorsoventricular ridge, external nucleus of the amygdala, medial POA, AH, lateral septum, dorsolateral anterior nucleus, VMH, periventricular nuclei of the hypothalamus, and especially the premammilary nucleus (Young *et al.*, 1994; Rhen and Crews, 2000). This distribution reveals differences from previously documented distributions in the brains of green anole lizards and garter snakes based on steroid autoradiography. Specifically, AR mRNA is more abundant than ER mRNA in the lateral septum, and ER mRNA does not occur in the external amygdala of whiptail lizards. Of particular interest from a comparative perspective is the expression of AR mRNA in the dorsoventricular ridge (DVR) of the lizard. The DVR appears homologous to the AR expressing magnocellular nucleus of the anterior neostriatum (MAN) in songbirds (Balthazart *et al.*, 1992). There are also some strong similarities between the patterns of AR expression in whiptail lizards and those in mammals.

Mapping AR gene expression by using *in situ* hybridization has also been employed in green anole lizards to examine the distribution of AR mRNA expression (Rosen *et al.*, 2000). These workers found AR mRNA in many of the same areas that express this message in the brain of whiptail lizards, with some differences. The regions of similarity include the POA, septum, amygdala, striatum, premammilary nucleus, VMH, torus semicircularis and brain-stem motor nuclei. In contrast to the patterns observed in whiptail lizards, AR mRNA expression was not localized to the DVR of green anole lizards in this study.

Moga and coworkers (2000) used an antibody directed at a conserved sequence in the N-terminal domain of the AR protein (residues 1–21 of the rat AR) to map AR immunoreactivity (AR-ir) in the fence lizard, *S. undulatus*. This method allows them to distinguish between staining in the nucleus and cytoplasm and also reveals AR-ir in axons and dendrites with a high anatomical specificity. AR-ir in the brain of *S. undulatus* showed good, but not complete, agreement with the distribution of AR determined in other species and by other methods previously described. AR staining was found in males in the medial and dorsal cortices, medial septum, and several cell groups in the amygdala as well as the adjacent bed nucleus of the stria terminalis. These investigators also found nuclear staining in the medial POA, periventricular hypothalamus, and ventromedial, premammillary, and arcuate nuclei. AR-ir fiber staining occurred throughout the AH and POA and in a variety of other diencephalic areas. Females showed nuclear AR-ir in fewer areas than males, with nuclear staining only in the ventroposterior amygdala and VMH (the ventroposterior amygdala appears homologous to the external amygdala of whiptail lizards based on anatomy and AR expression; Moga *et al.*, 2000). Fiber staining in male and female fence lizards was broadly similar. The sex difference in distribution found here for *S. undulatus* could represent a species difference or a difference in the sensitivity of the immunocytochemical method employed relative to the *in situ* hybridization approach used in other studies. Male whiptail lizards do show higher levels of AR mRNA in the medial POA (MPOA) than females (Godwin *et al.*, 2000). The results reported for fence lizards could be very similar if females do express AR mRNA in the MPOA, but they express AR protein at levels too low to be detected by immunocytochemistry. A difference in the sensitivity of the technique could also account for the lack of AR-ir observed in the dorsolateral thalamic nucleus and anterodorsal ventricular ridge, a result that also contrasts with the described distribution of AR mRNA in whiptail lizards (Young *et al.*, 1994).

As in other groups of vertebrates, reptiles show what Pfaff and coauthors termed a lawfulness of steroid receptor distribution in the brain (Pfaff *et al.*, 1994). Indeed, this conservation in distribution provides evidence of neural homologies, as with the expression of AR in the lizard DVR and the avian MAN. Sex steroid

receptors are widely distributed in areas of the brain that play critical integrative roles in sociosexual behavior, including the POAH and VMH. The identification of sites outside these areas expressing steroid receptors has been facilitated by the development of *in situ* hybridization techniques and antibodies directed at conserved regions of the receptor proteins. In contrast to what is known about mammals and fishes (Kuiper *et al.*, 1996; Hawkins *et al.*, 2000), there is no information available on different forms of the sex steroid receptors in reptiles. The *in situ* hybridization data presented for ER in lizard brain are for ERα (Young *et al.*, 1994). It is also not known whether the alternatively spliced form of the ER, Δ-4, identified in turtles, lizards, and rats shows a distribution in the brain that differs from the full-length form (Skipper *et al.*, 1993). *In situ* hybridization has proven very useful for determining sites of steroid receptor synthesis, but immunocytochemical work further exploring regions of steroid responsiveness has been limited due to the lack of immunoreactivity of most antibodies available (M. Gahr and D. Crews, unpublished). Studies in this area and immunocytochemical work exploring colocalization of different receptor types in cells will be particularly useful.

B. Regulation

The ability to localize and compare relative levels of abundance of the three main gonadal steroid hormone receptors in whiptail lizards has allowed a variety of questions related to sex and species differences in their regulation to be addressed. A key species difference in whiptail lizards is unisexuality and the display of male-like pseudocopulatory behavior by the parthenogenetic *C. uniparens*, but not by females of its sexual ancestor *C. inornatus* (Crews and Fitzgerald, 1980).

E_2 increases ER mRNA abundance in discrete brain regions in the whiptail lizards. Young and coworkers documented this using a 0.5-μg injection of estradiol benzoate (EB) and measuring ER mRNA abundance 24 hours after administration. The EB effectively stimulates female-typical receptive behavior in parthenogenetic whiptail lizards (Young *et al.*, 1995a), and it increases ER mRNA in some regions (torus semicircularis and VMH), decreases it in others (lateral septum), and causes no change in still other nuclei (periventricular nuclei of the hypothalamus, periventricular nucleus of the POA, and the dorsal hypothalamus). The increase seen in ER mRNA in the VMH is particularly interesting for three reasons. First, as previously mentioned, this nucleus critically regulates female-typical sexual behavior in both the sexual and unisexual parthenogenetic whiptail lizards. Second, the pattern of increased ER mRNA in the mediobasal hypothalamus is opposite that seen in rats, in which estrogen down-regulates its receptor. This difference between whiptail lizards and rats may relate to differences in the nature of their ovarian cycles. Whiptail lizards have elevated E_2 levels for a relatively long period prior to ovulation and display receptive behavior for the duration of this period, whereas female rats are receptive for only a short window following ovulation. Young and Crews (1995) suggest that prolongation of the time span E_2 levels are elevated and of sexual receptivity may be quite common in mammals (e.g., cats and rabbits). Last, species comparisons indicate that parthenogenetic whiptails have higher concentrations of ER mRNA expression in the POA than do sexually reproducing female whiptails (Young *et al.*, 1995b). This observation led in turn to the sensitivity compensation hypothesis (Fig. 22), which proposes that an inverse relationship exists between the expression of the genes coding for sex steroid hormone receptors in the POA and circulating concentrations of sex steroid hormone. The increased level of ER gene expression in the POA results in a greater sensitivity to the circulating concentrations of E_2 that, in turn, results in lower levels of circulating E_2 through feedback effects.

E_2 also stimulates increases in PR mRNA abundance in lizard brains, but again typically in a manner specific to species, sex, and region. Female green anoles show increases in progestin binding sites with estrogen treatment (Tokarz *et al.*, 1981) as well as induction of sexual receptivity (Tokarz and Crews, 1980). EB treatment strongly induces PR mRNA in the VMH of whiptail lizard females. The degree of this induction is tightly correlated with the display of female-typical receptive behavior in *C. inornatus* and female-like pseudosexual behavior in the parthenogenetic *C. uniparens* (Fig. 23) (Young *et al.*, 1995b), with EB being more effective in the parthenogenetic *C. uniparens*. EB also effectively stimulates increases in PR mRNA in the POA of female *Cnemidophorus* again with similar dosages being more effective in the parthenogen *C. uniparens* than

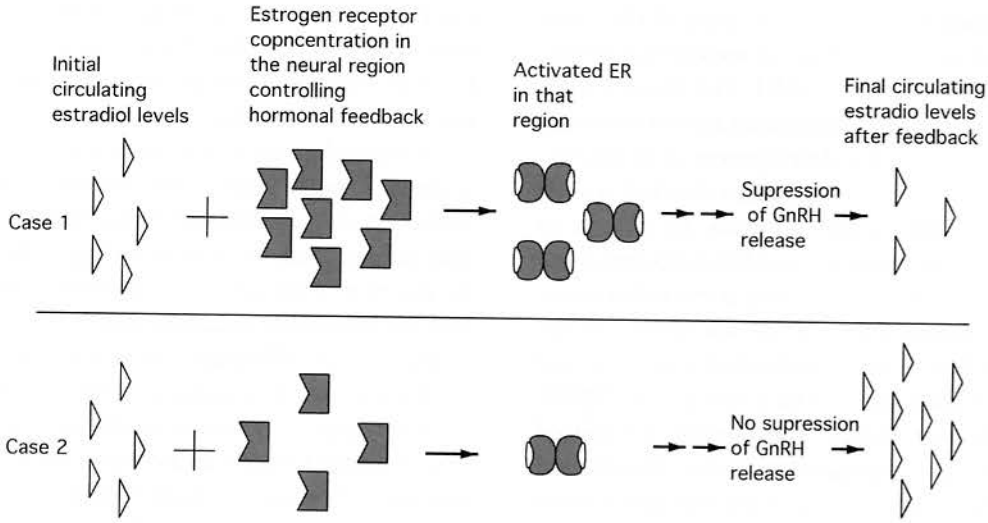

FIGURE 22 Schematic illustrating the sensitivity compensation model for species differences in the circulating concentrations of sex steroid hormones. Two cases (species) are illustrated that differ in the abundance of estrogen receptor (ER) in the neurons involved in the negative feedback loop. Under the initial conditions illustrated, both systems are presented with identical hormone concentrations. However, due to differences in the number of receptor molecules, the neurons in case 1 have more activated estrogen receptors, which results in an inhibition of gonadotropin-releasing-hormone (GnRH) release and ultimately a lower circulating concentration of hormone. In case 2, less activated receptors are formed, GnRH release is not inhibited significantly, and hormone levels remain the same or rise.

in females of the sexual ancestor *C. inornatus* (Godwin and Crews, 1999). This greater estrogen stimulation of PR mRNA in the brain region mediating male-like pseudosexual behavior in *C. uniparens* may be related to the display of male-like pseudosexual behavior by *C. uniparens*, but not by *C. inornatus* females.

Although E_2 increases PR mRNA in both the VMH and POA of female and parthenogenetic whiptail lizards, exogenous P inhibits both female-typical receptive behavior and decreases estrogen-stimulated ER and PR mRNA in the VMH (Godwin *et al.*, 1996). This effect of P on both receptivity and on ER and PR mRNA abundance is similar to patterns in well-studied rodent models (Blaustein and Turcotte, 1990; Blaustein *et al.*, 1994; Brown and Maclusky, 1994). In contrast, exogenous P has no effect on PR mRNA abundance in the periventricular POA in this experiment.

Neither the effective induction of female-typical receptive behavior nor increases in ER and PR mRNA in the VMH seen in female and parthenogenetic whiptail lizards occur in short-term castrate males (1 week) (Godwin and Crews, 1995). This lack of responsiveness to estrogen in the VMH of male whiptail lizards parallels patterns in rats (Lauber *et al.*, 1991a,b). In contrast, male *C. inornatus* castrated for longer periods (6 weeks) showed PR mRNA responses to estrogen that were not different from females (Wennstrom and Crews, 1998). Females implanted with T, however, did not show an attenuation of the female pattern of responsiveness. These results indicate that the maintenance of the male-typical pattern of nonresponsiveness requires the activational effects of T, whereas the female-typical pattern is less plastic.

The abundance of PR mRNA is also correlated to the display of male-typical sexual behavior in male *C. inornatus* (Crews *et al.*, 1996b). Male *C. inornatus* can be classified as either P-sensitive or P-insensitive based on the effectiveness of exogenous P delivered in Silastic capsules implanted intraperitoneally in reinstating sexual behavior following castration (Lindzey and Crews, 1992). Males classified as P-sensitive are also significantly more likely to respond to intracranial implants of P (directed at the POA) than are P-insensitive males (Crews *et al.*, 1996b). Interestingly, there are also

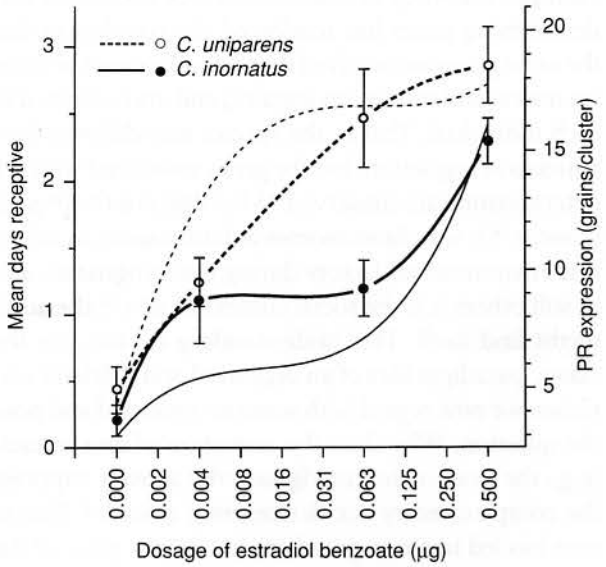

FIGURE 23 Species differences in the induction of sexual receptivity (thin lines) and progesterone receptor mRNA expression (bold lines) by estradiol benzoate (EB) in ovariectomized whiptail lizards. Ovariectomized animals were given a single injection of EB and either were tested daily for receptivity for 4 days following the injection or had their brains removed 24 hours after treatment and analyzed using *in situ* hybridization. Vertical error bars represent standard errors of the mean.

differences in both PR and AR mRNA abundance between the two groups following intracranial implantation of P. Progesterone-sensitive males display lower abundances of PR mRNA in both the medial and periventricular portions of the POA, but higher abundances of AR mRNA in the medial POA, external amygdala, and lateral septum. No differences are seen between P-sensitive and P-insensitive males without an intracranial implant.

Sex and species differences are also found in the androgenic regulation of ER, PR, and AR mRNA. The implantation of gonadectomized male and female *C. inornatus* and parthenogen female *C. uniparens* with either T or DHT reveals a diversity of effects, suggesting that gonadal sex, aromatization, and gene dosage (ploidy) all influence steroid receptor mRNA response (Godwin *et al.*, 2000; see also Young *et al.*, 1995a, for PR mRNA). For example, males have higher AR mRNA in the MPOA than females of either species and these levels decrease with T treatment in males, but not in females. In contrast, ER and PR mRNA levels in the VMH are higher with androgen treatment, but these effects do not differ by sex. There also are species effects in that the triploid parthenogen shows higher steroid receptor mRNA abundances overall than the diploid sexual females. Finally, aromatization of T to estrogen is probably important in some regions. PR mRNA in the periventricular POA is increased in both males and females by T, but not by nonaromatizable DHT.

Last, individual experiences might influence gene expression in the brain directly rather than via the modulation of the endocrine physiology of the partner. For example, in the hamster and the rat, exposure to the sexual behavior of the opposite sex induces expression of the immediate to early gene c-*fos* in those brain regions that mediate sexual behavior (see Chapters 1, 2, and 15). Using ovariectomized, hormone-primed, parthenogenetic whiptail lizards, Hartman and Crews (1996) demonstrated that participating either as a male or as a female during a pseudosexual encounter significantly alters the abundance of ER and PR mRNA in the hypothalamus of whiptail lizards.

In contrast to the conservation of steroid receptor distribution in the brain in reptiles and other vertebrates, the patterns of steroid receptor regulation vary greatly. Studies in lizards show that steroid receptors in the brain are regulated by both their own ligands and other steroid hormones. This regulation shows variation across brain nuclei, both within and between the sexes, between closely related species, and with social interactions. Some of the patterns found are strikingly similar to those seen in well-studied rodent models, but there are also differences that appear to be related to differences in the nature of the reproductive cycles. It is important to note that most of the studies examining steroid receptor regulation have either shown behavioral effects or used behaviorally relevant dosages of hormone, supporting a role for this regulation in behavioral display. Remaining challenges in this area include determining the degree of colocalization of receptor types in neurons and cross talk between signalling systems, exploring the influences of other mediators (e.g., corticosteroids, thyroid hormones, and neurotransmitters) on receptor regulation, and characterizing the downstream effects of steroid receptor activation.

VII. CONCLUSION AND FUTURE DIRECTIONS

Reptiles enable the study of the neuroendocrine mechanisms underlying sociosexual behaviors in ways not possible with conventional animal model systems. This work has had two important impacts on our understanding of sociosexual behavior. First, it has revealed the great diversity that exists among vertebrates in reproductive behaviors and the neuroendocrine mechanisms underlying these behaviors. For example, the study of species with dissociated reproductive tactics and unisexual species has suggested three factors that may explain species differences in endocrine physiology and behavior: (1) sensitivity to sex steroid hormones, (2) hormone-dependent regulation of sex-steroid-hormone-receptor gene expression, and (3) neuroanatomical distribution of steroid receptor gene expression, especially in nonlimbic structures.

The second major impact arises from explorations of this diversity within and across major taxa. These explorations allow us to begin defining which mechanisms show strong conservation and which are evolutionarily more labile. Reptiles and mammals diverged approximately 300 million years ago, yet research in reptiles has revealed the apparent conservation of many behavior-controlling mechanisms between these groups. For example, research in reptiles has led us to reexamine certain assumptions in behavioral neuroendocrinology—for example, that progesterone is a female-specific hormone with no function in males. Experiments with four lizard species demonstrated that progesterone is vital to the display of male copulatory behavior in lizards and, further, that androgen and progesterone synergize in males much like estrogen and progesterone synergize in females to facilitate sexual receptivity; subsequent studies with mice and rats have revealed similar roles for progesterone and its receptor in male sexual behavior in male mammals. Continuing to identify those mechanisms that are fundamentally important in all vertebrates and those that represent axes along which evolutionary change may take place will lead to a more complete understanding of the diversity we see and how this diversity arose.

Research in reptiles has also contributed and continues to contribute to our understanding of animal sexuality and the nature of individual variation. For example, the study of animals that lack sex-linked sex-determining genes has reinforced the conclusion that the same genes are involved in the development of testes (in males) and ovaries (in females) and are contained in each individual. That is, the species may differ in their patterns of regulation, but the genes associated with sex determination are conserved. What differs is the trigger; in some it is sex chromosomes at fertilization, in others it is environmental factors during embryogenesis, and in still others it is the social context in which the adult might find itself. This understanding is changing the classic paradigm idea of an organized and a default sex; rather, we now regard both sexes as organized and pose the question, Why does the activation of one cascade (e.g., the ovary-determining cascade) actively suppress the complementary sex-determining cascade? This in turn has led to a new paradigm to take the place of the organized–default paradigm, namely that the female is the ancestral sex and the male the derived sex. A logical extension of this paradigm is the question, Why might males be more like females (rather than females being like males)?

The mechanisms that generate individual variation are an important focus across modern biology. Understanding these mechanisms is of fundamental importance for addressing basic issues, such as how evolutionary change takes place, and applied problems in human health. Research in reptile behavioral neuroendocrinology has contributed to our understanding of behavioral variation, particularly as it relates to sexually dimorphic behaviors. For example, it has been proposed that some behavioral sex differences in mammals may be mediated directly by expression of *Sry* in the brain (see Chapter 63). Evidence of direct genetic influences on the organization of behavior was first proposed in studies of nonmammalian vertebrates that completely lack sex chromosomes—the neural organization underlying sex-typical behaviors may depend on behavioral or physical stimuli in the environment (Crews, 1994). Sex-changing fish typify the first, the social environment effectively switching the brain and behavior, and ultimately the gonad, from one sex to the other (Godwin *et al.*, 2000). Dependence on the second stimulus is characteristic of reptiles that exhibit TSD in with the temperature of the incubating egg in the mid-trimester of embryonic development determining gonadal sex. Another example of this idea

comes from studies of parthenogenetic, or all-female, whiptail lizards. These unique animals arose from the hybridization of sexually reproducing species and sex chromosomes appear to exist in the ancestral sexual species with male heterogamety (XY). The expected sexual dimorphisms are present in morphology, physiology, brain anatomy, and behavior, all of which are under testicular hormone control. In the descendant unisexual species, however, no males exist and all individuals have a female phenotype. Remarkably, these parthenogens reliably and regularly exhibit both male-like and female-like pseudosexual behaviors during the course of their reproductive cycle. Although males do not exist, the gene(s) for male development have not been lost but, instead, appear to be repressed. Although the genetic trigger for male development (Y) is absent, the male-determining cascade can be activated by treating embryos with aromatase inhibitor, thereby producing fully functional males. Such animals exhibit only male-like copulatory behavior and are insensitive to exogenous estrogen. However, their brain anatomy remains similar to that of normal parthenogens who, despite the bisexual nature of their behavior, have strictly female-like brain morphology. Thus, the expression of Y chromosome gene products in whiptail lizards not only influences brain anatomy, but suppresses the display of female-like behavior and sensitivity to exogenous estrogen.

Many challenges remain in the study of hormones, brain, and behavior in reptiles. Nearly all the information available regarding the hormonal and neural bases of behavior in reptiles comes from studies of lizards and snakes. Although this gives insight into these mechanisms in the most speciose group of reptiles, little is known about hormone–brain–behavior relationships in the other major lineages of reptiles, the turtles and crocodilians. Modern birds represent the most derived forms in the archosauromorph lineage, with crocodilians being the most primitive and the extinct dinosaurs falling in between. Our understanding of behavioral mechanisms in birds would benefit from a more thorough understanding of these mechanisms in the primitive members of the lineage, the crocodilians.

The lack of correspondence between structure of the nervous system and behavioral phenotype highlights the need for more comparisons of a functional nature. Insights from measurements of neural metabolic activity and capacity, neurotransmitter metabolism and influences, and the regulation and actions of steroid hormone receptors all show the value of these approaches.

The diversity of patterns in sex determination and differentiation seen in reptiles has provided important evidence that factors other than gonadal steroid hormones can have critical influences on the differentiation of the neural substrates of behavior. Elucidating these influences and the interplay of factors such as temperature and social interactions with gonadal steroids in shaping the function of the adult nervous system is an important research direction.

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