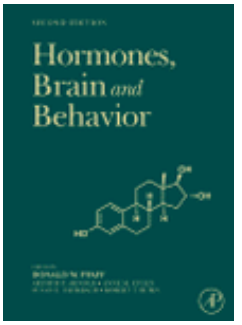

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23 Hormones, Brain, and Behavior in Reptiles

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Glossary

associated reproductive pattern A pattern of reproductive behavior in which animals exhibit sexual behavior when their gonads are actively producing gametes and their steroid hormone levels are elevated.

constant reproductive pattern A pattern of reproductive behavior in which reproductive readiness is maintained, but actual reproduction and display of sexual behavior are limited to short periods when environmental conditions are appropriate.

dissociated reproductive pattern A pattern of reproductive behavior in which the display of sexual behavior is temporally uncoupled from gamete production.

heterogamety The production of unlike gametes by an individual of one sex.

pseudocopulatory behavior Display of male-like copulatory behavior by a female.

relative plasticity hypothesis A proposal that fixed differences between alternate reproductive phenotypes reflect organizational actions of gonadal steroid hormones while plastic differences reflect activational effects of gonadal steroid hormones.

temperature-dependent sex determination Pattern characteristic of many species of reptile in which gonadal sex is determined by the temperature experienced by the developing embryo.

23.1 Introduction

The living group of animals we refer to as reptiles includes essentially all the amniote vertebrates except the mammals and the birds. It is represented by four living orders: the Squamata (lizards, snakes, and amphisbaenids), the Testudines (turtles and tortoises), the Crocodilia (crocodiles, alligators, etc.), and the Sphenodontia (tuataras) (Figure 1). In order to make this taxon monophyletic, it would be necessary to include the approximately 10 000 species of birds (Padian and Chiappe, 1998), but for the purpose of this chapter, discussion is limited to the familiar reptile forms such as lizards, snakes, turtles, and crocodilians. These animals offer two large advantages from an experimental standpoint. The first of these is the enormous diversity of patterns of sexual differentiation observed across reptilian species, enabling some particularly illuminating natural experiments, of which several are discussed below. Among the diverse patterns represented are temperature-dependent sex determination (TSD), reproduction by obligate parthenogenesis (all-female species), and distinct alternate male phenotypes within a species. The second is the presence of many primitive (in the phylogenetic sense) characters in reptiles. Lastly, mammals and birds arose from turtle-like and crocodilian-like ancestors, respectively, allowing for phylogenetic comparisons.

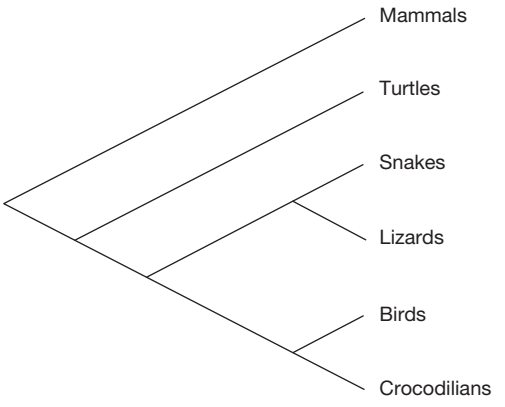

Figure 1 Phylogeny of amniote vertebrates. Mammals are believed to have arisen from turtle-like therapsid reptiles approximately 350 million years ago and modern birds from crocodilian-like archosaurs approximately 250 million years ago.

This chapter discusses how these advantages have been exploited to yield new insights into the nature of the neuroendocrine control of behavior in vertebrates in general. We begin with a short review of diversity in sex determination, sexual differentiation, and hormone–behavior relationships observed in reptiles. This is followed by discussion of some of the neural mechanisms involved and the methods that have been successfully applied to elucidating them. In each of these sections we focus equally on within-sex and between-sex differences in the neurochemistry of brain areas subserving sexual behavior in reptiles. The diversity of reproductive patterns in reptiles enables some comparisons that are not possible with mammals or birds and so extends these findings. Finally, we summarize some research directions that are likely to prove especially promising.

Modern reptiles exhibit phenotypes that are in many ways similar to what 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, makes modern reptiles useful 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 regulation of steroid hormone receptors are common. Further, 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 that modern reptiles likely 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 (GSD) that is displayed within 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 in sex determination and sexual differentiation (Crews and Gans, 1992). **Table 1** presents examples of some of the questions that reptiles are particularly useful in addressing. Further, such comparative studies have additional benefits. First, some phenomena heretofore unrecognized in other amniote groups are evident in reptiles, leading to renewed study in mammals, and increasing 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 sufficient numbers of species, including outgroups, to generate adequate sample size for comparisons. For example, viviparity has evolved from oviparity, perhaps 100 or more times independently in reptiles (Blackburn, 1999; Guillette, 1991).

The lack of a well-developed cortex in reptiles is experimentally advantageous in many ways. Greenberg et al. (1979) refer to reptiles as walking limbic systems and note the value of interpretations not being subject to the complications presented by a well-developed cortex. Modern reptiles are likely also primitive in the neural circuitry that mediates mounting and 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 how they function in birds and mammals.

23.2 Diversity in Sex Determination, Sexual Differentiation, and Hormone–Behavior Relationships

Reptiles show an extraordinary diversity of sex determination and differentiation patterns. In addition to GSD, many reptiles possess primitive (e.g., TSD) or specialized (e.g., obligate parthenogenesis and alternative mating tactics) traits that have added new dimensions to our understanding of reproductive neuroendocrine mechanisms underlying reproduction in vertebrates in general. One important benefit

Table 1 Questions and possible comparisons using reptiles as model systems

Questions	*Comparison*	*Example*
How do behaviors and their underlying brain mechanisms change in speciation?	Ancestor vs. descendant species	*Cnemidophorus inornatus* vs. *C. uniparens*
What is the effect of ploidy on neuroendocrine mechanisms?	Diploid vs. triploid species	*Cnemidophorus inornatus* vs. *C. uniparens*
Are there functional differences in genotypic sex-determining systems?	Male heterogamety vs. female heterogamety	*C. inornatus* vs. *Thamnophis sirtalis parietalis*
Are there functional differences in genotypic sex-determining systems (GSD) vs. temperature-dependent sex-determining (TSD) systems?	GSD lizard vs. TSD lizard	*C. inornatus* vs. *Eublepharis macularius*
	GSD turtle vs. TSD turtle	*Trionyx spniferus* vs. *Trachemys scripta*
Causal mechanisms and functional outcomes of different phenotypes of the same sex?	Alternative reproductive patterns	*Urosaurus ornatus* vs. *Uta stansburiana*
What has the environment altered in the functional relationship between gamete growth, sex steroid hormone secretion, and mating behavior?	Associated vs. dissociated reproduction	*Anolis carolinensis* vs. *Thamnophis* spp.

of 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, 1998a). In contrast, many reptiles show more continuous variation in these behaviors. Examples include species with TSD that show substantial behavioral variation within sexes across incubation temperatures, all-female species in which individuals alternate between the display of female- and male-like pseudosexual behavior during the course of the ovarian cycle, 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. Our goal in this section is to highlight diversity in reptilian sex determination and differentiation patterns and the research opportunities this diversity presents.

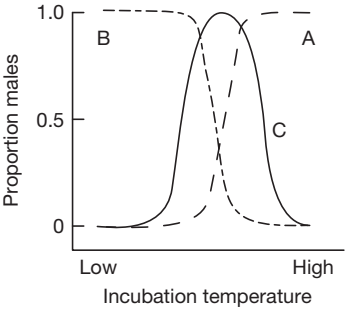

Figure 2 Response of hatchling sex ratio to incubation temperature in various egg-laying reptiles. These graphs represent only the approximate pattern of the response and are not drawn according to any single species. The three patterns recognized presently are (A) only females produced from low incubation 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.

23.2.1 Temperature-Dependent Sex Determination

Many reptiles exhibit GSD, with some species exhibiting male heterogamety (XX:XY) like mammals, while others exhibit female heterogamety (ZZ:ZW) like birds. However, in all crocodilians (alligators, crocodile, caiman, etc.), most turtles (e.g., all marine turtles and tortoises and many freshwater turtles), and some lizards (e.g., various Geckkonid and Agamid species), gonadal sex is established by the temperature experienced by the incubating egg (Viets et al., 1994; **Figure 2**). Once thought to be restricted to oviparous species lacking sex chromosomes, recent reports have extended TSD to oviparous and viviparous species with both XX:XY and ZW:ZZ sex-determining systems (Robert and Thompson, 2001; Shine et al., 2002; Quinn et al., 2007).

23.2.1.1 Mechanisms of TSD

Three basic patterns of TSD have been documented (**Figure 2**; Valenzuela and Lance, 2004). In the first pattern, relatively high incubation temperatures produce males, whereas relatively low temperatures produce females. The second pattern is simply the reverse; and the third is a more complex sex-determining pattern in which intermediate temperatures produce males and high and low temperatures produce females. Sensitivity to temperature is restricted to the mid-trimester of development, or the temperature-sensitive period (Crews, 1996a, 2003). TSD is believed to be ancestral to the GSD characteristic of birds and mammals (Crews, 1994).

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; Pieau and Dorizzi, 2004; Sarre et al., 2004; Wibbels et al., 1998). For example, 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). Similarly, male sex determination can be manipulated by exogenously applied dihydrotestosterone (DHT), a nonaromatizable androgen, and its derivatives, as well as by reductase inhibitors (Crews et al., 1996a). Importantly, temperature and hormones act synergistically, further indicating that steroid hormones are undoubtedly a part of TSD in both males and females (Crews, 1996a; Crews et al., 1994, 2006; Wibbels et al., 1991).

Recent research has begun to investigate the molecular mechanisms that underlie TSD, focusing on the genes involved in the events downstream from the temperature-induced trigger, as yet unknown. The same genes involved in gonadal differentiation

in mammals and birds are involved in the ovary- and testis-determining cascade in TSD (Fleming and Crews, 2001; Kettlewell et al., 2000; Maldonado et al., 2002; Murdock and Wibbels, 2006; Ramsey and Crews, 2007a,b; Ramsey et al., 2007; Rhen et al., 2007; Shoemaker et al., 2007a,b; Valenzuela and Shikano, 2007; Valenzuela et al., 2006; Yao et al., 2004).

23.2.1.2 Organizing influence of incubation temperature in the leopard gecko

Animals with environmental sex determination, such as lizards with TSD, are particularly suitable for developmental studies designed to distinguish between genetic and hormonal influences on adult sexual behavior. The leopard gecko (*Eublepharis macularius*), in particular, has proven to be an excellent model because the investigator has precise control of the critical environmental variable (in this case, incubation temperature) that determines the sexual phenotype of the gonad, and hence its products. Because these animals exhibit the third pattern of sex determination (discussed above), the sex ratio varies with temperature, but individuals of both sexes are produced at most incubation temperatures. By incubating eggs at these 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; Sakata and Crews, 2004a).

In the leopard gecko, incubation of eggs at 26 °C produces only female hatchlings, whereas incubation at 30 °C produces a female-biased sex ratio, and incubation at 32.5 °C produces a male-biased sex ratio; incubation of eggs at 34–35 °C again produces virtually all females (**Figure 3**). Hence, females from eggs incubated at 26 °C are referred to as low-temperature females, whereas 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 within females, those from a male-biased temperature have wider heads than do those from a low

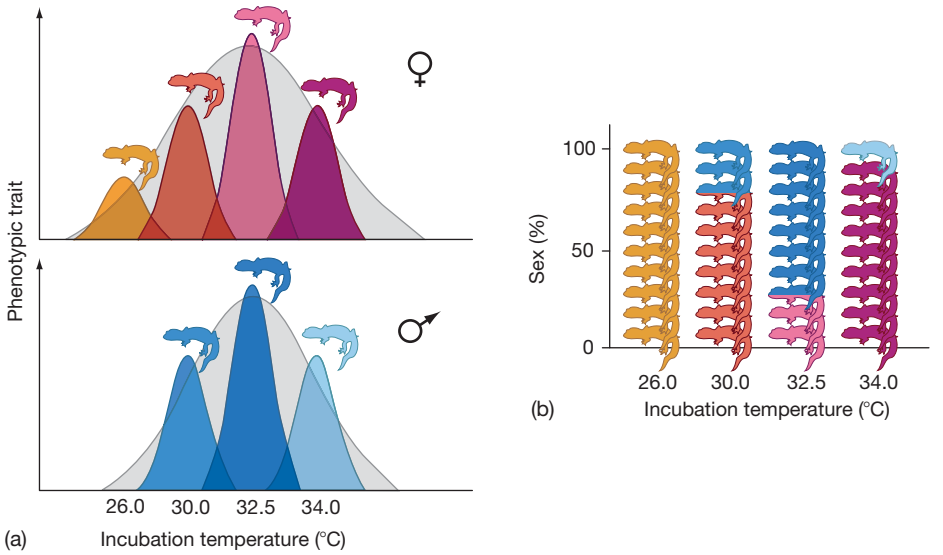

Figure 3 Pattern of temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. (b) Portrays the effect of incubation temperature on sex ratio: extreme temperatures produce females, whereas intermediate temperatures produce different sex ratios. Since the effects of incubation 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. By studying same-sex animals that differ only in the incubation temperature experienced reveals the effects of temperature (a), whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex.

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 a female-biased temperature, and become as large as males from a female-biased temperature (Tousignant and Crews, 1995).

In TSD reptiles, administration of exogenous estrogen to the incubating egg early in development will overcome a male-determining temperature effect and results in females; the opposite will occur if an aromatase inhibitor is administered to an egg incubating at a female-producing temperature, which results in male offspring. Using this method, it is possible to separate the direct action of temperature on brain organization from that arising from the type of gonad that is formed. Such studies reveal that some traits are influenced by temperature, others by gonadal secretions, and still others by a mixture of the two. For example, female leopard geckos from estrogen-treated eggs incubated at the male-biased temperature do not differ in growth rates from unmanipulated females from the same temperature, indicating that it is incubation temperature, and not hormones, that regulates body growth.

At hatching, circulating concentrations of sex hormones are already different between males and females, and this sex difference increases throughout life until, as adults, concentrations of testeosterone in males are approximately 100 times higher than in adult females (Gutzke and Crews, 1988; Tousignant and Crews, 1995; Rhen et al., 2005). However, the endocrine physiology of the adult varies in part due to the temperature experienced during incubation (Coomber et al., 1997; Tousignant et al., 1995; **Figure 4**). For example, plasma estrogen levels are significantly higher in males from a female-biased temperature compared to males from a male-biased temperature. Among females, circulating estrogen levels are significantly higher, and androgen levels significantly lower, in low-temperature females compared to females from a male-biased temperature.

Incubation temperature also has a major influence on the nature and frequency of the behavior displayed by the adult leopard gecko. Females usually respond

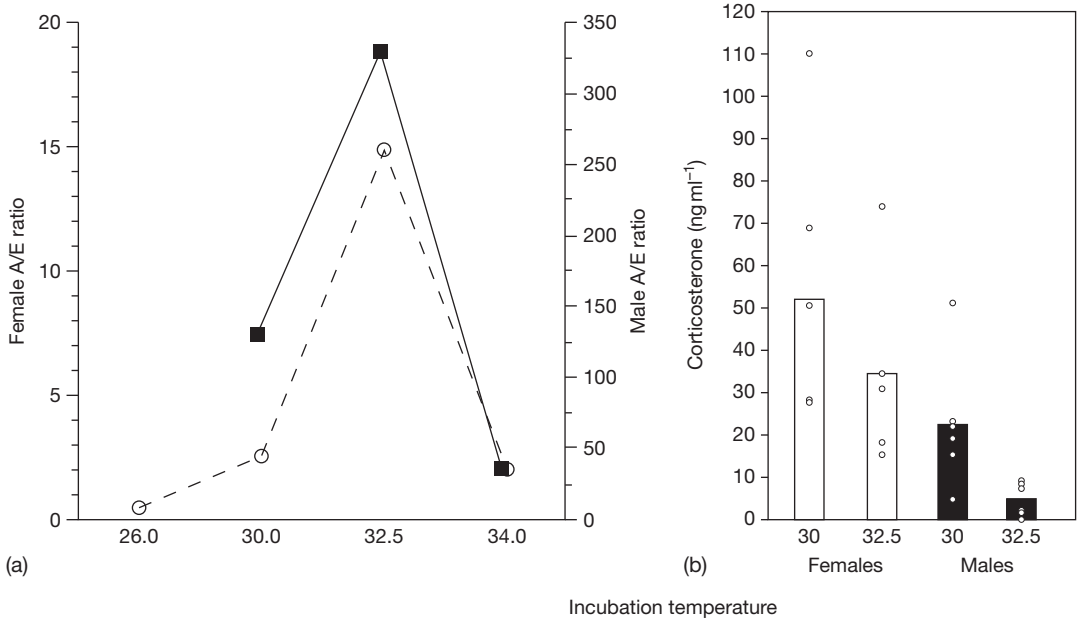

Figure 4 Circulating levels of steroid hormones vary between males and female leopard geckos (*Eublepharis macularius*) as well as between individuals from different incubation temperatures. (a) Ratio of the plasma levels of total androgens (A) and estrogens (E) in adult female (dashed line) and male (solid line) leopard geckos from different incubation temperatures. (b) Circulating concentrations of corticosterone (individuals indicated by circles). Data from Coomber P, Crews D, and Gonzalez-Lima F (1997) Independent effects of incubation temperature and gonadal sex on the volume and metabolic capacity of brain nuclei in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *Journal of Comparative Neurology* 380: 409–421; Gutzke WHN and Crews D (1988) Embryonic temperature determines adult sexuality in a reptile. *Nature* 332: 832–834; and D Crews, unpublished.

aggressively only if attacked, whereas males will 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 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 testosterone to induce 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 do males from the female-biased embryonic temperature when treated with DHT or testosterone; treatment with estrogen decreases submissive behavior in males from a male-biased embryonic temperature compared to males from a female-biased embryonic temperature (Rhen and Crews, 1999; **Figure 5**). Lastly, geckos from different incubation temperatures exhibit significant differences in dopaminergic activity (Dias et al., 2007). 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 will slowly approach 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. If, however, ovariectomized females from low and male-biased temperatures are treated with testosterone, they will 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 with females, incubation temperature influences sensitivity to exogenous hormones in males. Estrogen treatment will induce receptive behavior in castrated

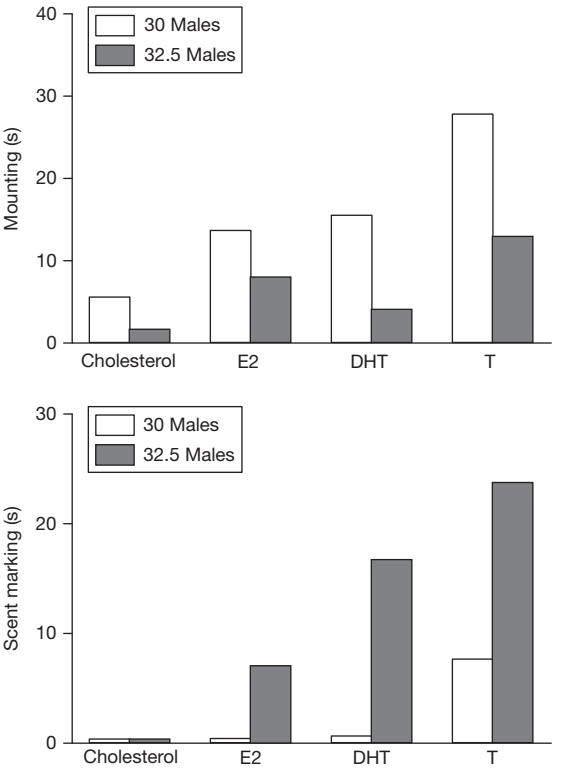

Figure 5 Incubation temperature influences sensitivity to exogenous hormones in adult male leopard geckos (*Eublepharis macularius*). Illustrated are the effects of embryonic incubation temperature and adult hormone treatment on mounting (upper panel) behavior and scent marking (lower panel) of castrated male leopard geckos. Individuals received a Silastic implant containing cholesterol, estradiol-17β (E2), dihydrotestosterone (DHT), or testosterone (T). Data from Rhen T and Crews D (1999) Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination. *Endocrinology* 140: 4501–4508.

males if they were incubated at a female-biased temperature, but not if they were incubated at a male-biased temperature. Mate preference is also influenced by incubation temperature, with males from a male-biased temperature preferring to associate with females from a lower incubation temperature, while males from a female-biased incubation temperature prefer females from a higher incubation temperature (**Figure 6**).

As might be predicted, these behavioral differences among and between male and female leopard geckos from different incubation temperatures are reflected by differences in the neural substrates regulating these behaviors, including the size and metabolic activity of different limbic areas. One exciting development in our ability to assess sex differences in the neural substrates of sexual behavior has been the introduction of metabolic mapping techniques using cytochrome oxidase histochemistry (Sakata et al., 2005). Cytochrome oxidase 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).

As with brain nucleus volume, a variety of factors influence metabolic capacity in the brains of leopard

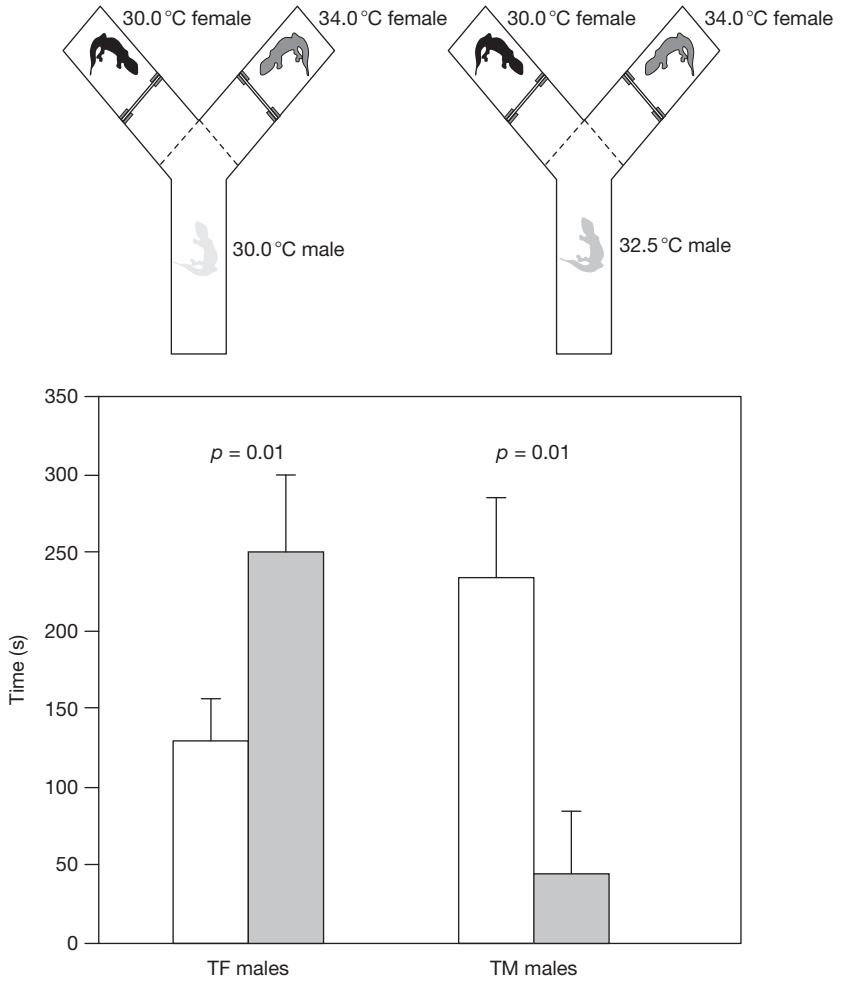

Figure 6 Differential mate choice behavior in male leopard geckos from two incubation temperatures. Individuals were placed in a Y-maze (top panel) where they could see, hear, and smell the stimulus females. Males from a male-biased incubation temperature (TM) spend a significantly greater amount of time close to females from a female-biased incubation temperature (TF) than with females from a high incubation temperature (TH) (bottom right panel), while the opposite was true for TF males (bottom left panel). Data from Putz O and Crews D (2006) Embryonic origin of mate choice in a lizard with temperature-dependent sex determination. *Developmental Psychobiology* 48: 29–38.

geckos. These include incubation temperature, age, and sexual experience. Incubation temperature affects cytochrome oxidase 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 (DVR), nucleus sphericus (NS), lateral septum (LS), and striatum, but do not increase capacity in the posterior hypothalamus or periventricular preoptic area (POA; **Figures 7** and **8**). Of particular interest is the finding that young females from a male-biased incubation show greater cytochrome oxidase (CO) levels in the medial POA (mPOA) than young females from a female-biased 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 ventromedial hypothalamus (VMH) than males from a male-biased incubation temperature (Sakata and Crews, 2004b). 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, NS, 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 (Coomber et al., 1997; Sakata and Crews, 2003a, 2004a,b; Sakata et al., 2000, 2002). Several nuclei, including the VMH and AH, have higher metabolic capacities in sexually experienced males compared to 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 compared to sexually inexperienced females regardless of incubation temperature history, while the results were mixed for other nuclei.

Sakata et al. (2000) assessed functional connectivity among limbic nuclei in the leopard gecko by analyzing covariance patterns in metabolic capacity, as revealed by quantitative CO histochemistry (**Figure 9**). As 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 aggression 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 dorsal ventricular ridge and septum are 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 (Crews et al., 2006; **Figure 10**).

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.

23.2.2 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 (Crews, 1984, 1987). The three elemental components of the reproductive process in vertebrates are gametes, steroid hormones, and behavior. In species in which the pattern of gonadal activity is associated temporally with mating, as occurs in many mammals and birds, these elements are also functionally associated. This has led to the long-standing assumption that increasing levels of gonadal steroid hormones in the

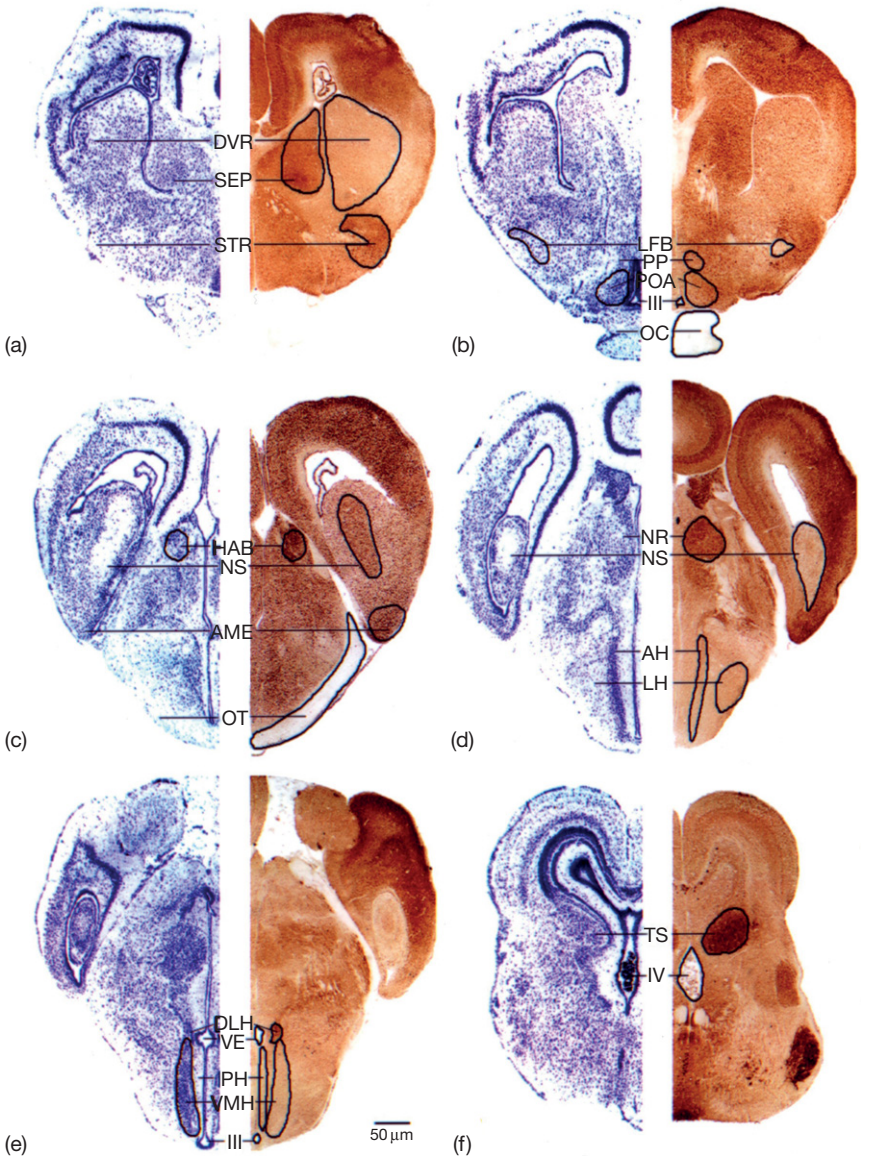

Figure 7 Adjacent sections from the leopard gecko (*Eublepharis macularius*) stained with cresyl violet (left) or histochemically stained for cytochrome oxidase (CO) activity (right); coronal sections with dorsal at the top and medial to the center of each picture. The brain depicted is from a 1-year-old male from an egg incubated at a male-biased temperature. (a)–(f) represent a rostral–caudal series of sections. Boundaries of the brain areas measured are traced. In left panels the areas measured for volumetrics are traced, whereas in the right panels the areas measured for CO activity are traced. Scale bar = 50 μm. Photographs are digitally processed. AH, anterior hypothalamus; AME, external amygdala; DLH, dorsal lateral nucleus of the hypothalamus; DVR, dorsal ventricular ridge; HAB, habenula; LFB, lateral forebrain bundle; LH, lateral hypothalamus; NR, nucleus rotundus; NS, nucleus sphericus; OC, optic chiasm; OT, optic tract; PH, periventricular nucleus of the hypothalamus; POA, preoptic area; PP, periventricular nucleus of the preoptic area; SEP, septum; STR, striatum; TS, torus semicircularis; VE, ventricular ependymal organ; VMH, ventromedial nucleus of the hypothalamus; III, third ventricle; IV, fourth ventricle. Reproduced from Crews D et al. (1998) Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. *Comparative Biochemistry and Physiology, C* 119:229–241, Copyright (1998) Elsevier.

circulation activates mating behavior. That is, there is a fundamental functional association among these three basic components. However, studies indicate that the dependence of mating behavior on sex hormones depends upon the reproductive pattern exhibited that, in turn, depends upon various ecological, phylogenetic, developmental, and physiological constraints on the organism. Beginning with studies

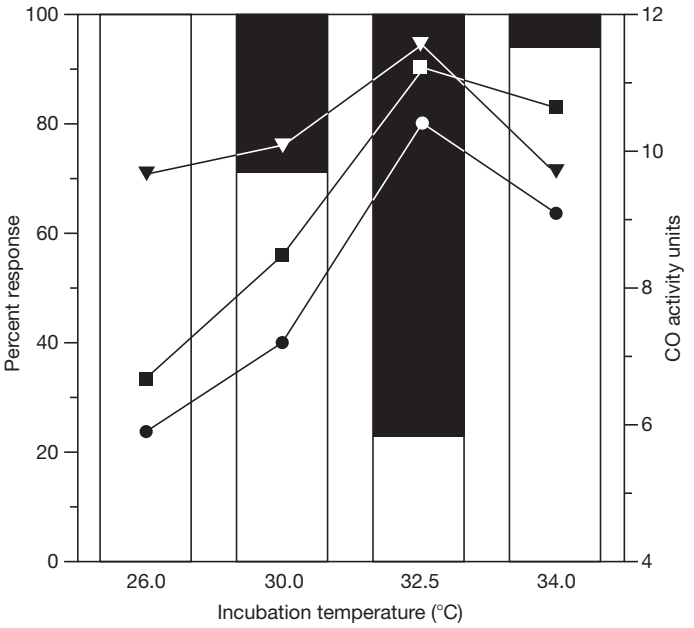

Figure 8 Relationship between sex ratio, aggressive behavior, and cytochrome oxidase (CO) activity in the amygdala in female leopard geckos. Sex in the leopard gecko is determined by the incubation temperature of the egg; the sex ratio produced at the temperatures indicated is reflected in the bar graph. The proportion of females responding aggressively toward a courting male indicated by squares. Inverted triangles and circles represent CO activity in amygdaloid nuclei of females from these same incubation temperatures. Thus, embryonic experience with temperature affects the level of aggressive behavior and brain metabolism in the amygdala of adult females. Reproduced from Godwin J and Crews D (2002) Hormones, brain and behavior in reptiles. In: Pfaff D, Etgen A, Arnold A, Fahrbach S and Rubin R (eds) *Hormones, Brain, and Behavior*, 1st edn. pp 545–585. San Diego, CA: Academic Press, with permission of Elsevier.

of reptiles (Crews, 1984; Crews et al., 1984) and more recently with 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 of sexual behavior. A number of studies now have shown that sexual behavior need not depend upon 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. 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, 1984, 1987). An interesting question is whether the other associations are derived and evolved independently in the different genera, and therefore are analogous, or were present in the common ancestor of each class (e.g., mammals) and therefore are homologous. This will only be determined through comparative analysis.

The display of reproductive behavior in reptiles and other vertebrate groups shows one of the three basic temporal relationships to gamete production (**Figure 11**). The most common relationship is termed as 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 termed as dissociated reproductive pattern. The most thoroughly studied reptilian example of this pattern remains the red-sided garter snake, *Thamnophis sirtalis parietalis* (Crews, 1983). This species is discussed at length below.

The third possible temporal relationship between gametogenic activity and reproductive behavior is a constant reproductive pattern. This pattern is characterized by 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

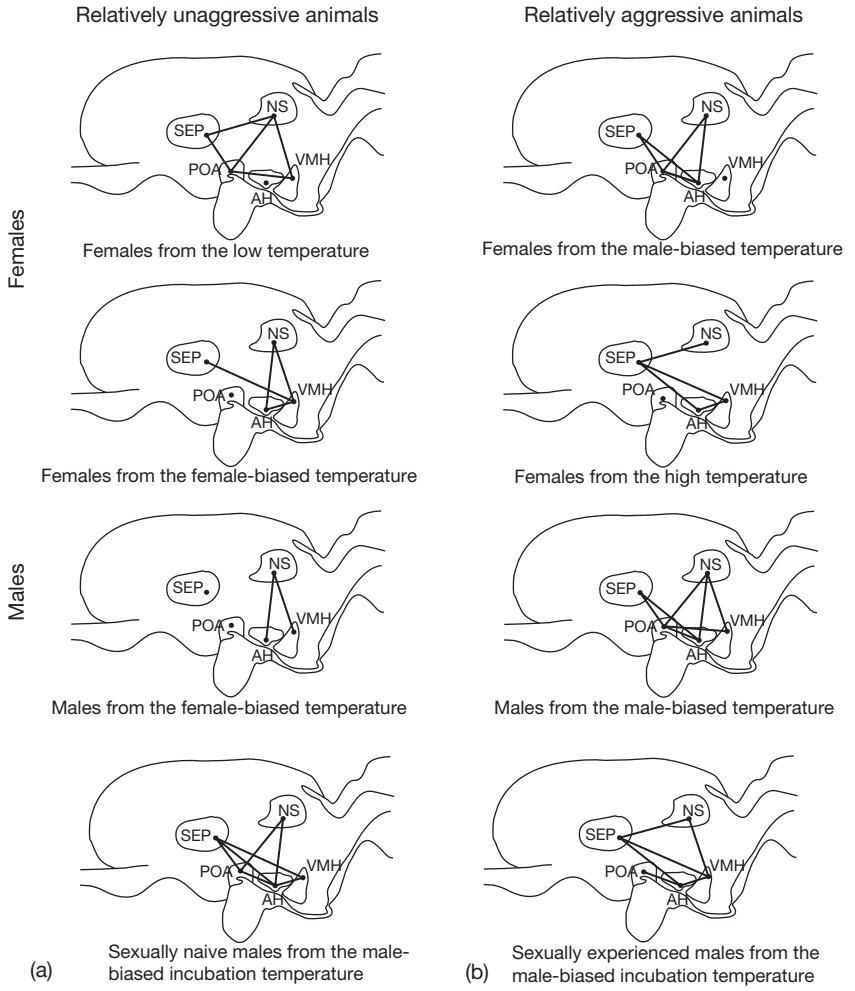

Figure 9 Incubation temperature and adult experience shapes the functional connections between limbic areas in the leopard gecko. Present are diagrams of the significant correlations among the anterior hypothalamus (AH), nucleus sphericus (NS), preoptic area (POA), septum (SEP), and ventromedial nucleus of the hypothalamus (VMH). All correlations are positive. (a) Note that the correlation between the AH and SEP is significant only in relative aggressive individuals of both sexes. (b) Note how sexual experience as an adult can modify the connections imposed by incubation temperature.

when environmental conditions are such that reproduction can be successful. While 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.

23.2.3 Alternative Mating Strategies

Many species of reptiles show alternate male phenotypes that exhibit discontinuous variation in male morphology, physiology, and behavior (Moore, 1991; Rhen and Crews, 2002). Alternative male phenotypes are also found in other vertebrate groups, including fishes, amphibians, birds, and in 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. As with the leopard gecko with TSD, such systems have the advantage of allowing comparisons of differing behavioral phenotypes without the confound of a difference in gonadal sex (Moore, 1991; Rhen and Crews, 2002). 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.

The behavioral and ecological correlates of alternative reproductive phenotypes in reptiles are better

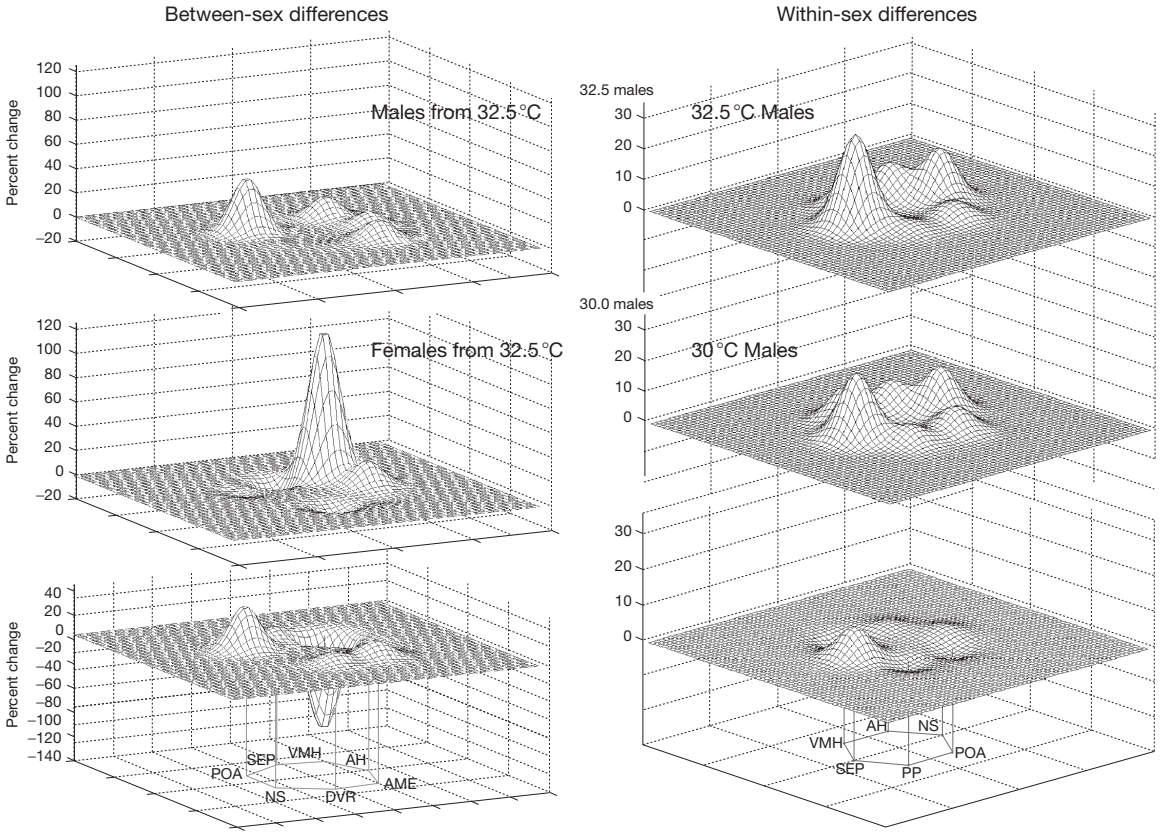

Figure 10 Between-sex and within-sex differences in metabolic capacity and in interconnected limbic nuclei of adult leopard geckos. Left panel depicts the limbic landscapes of males and females both from a 32.5 °C incubation temperature; the bottom graph indicates the difference between the neural landscapes of the sexes. Positive peaks indicate nuclei that change more positive in males, whereas negative peaks indicate nuclei in which change was more positive in females. Right panel depicts the limbic landscapes of males, the top graph of males from a 32.5 °C incubation temperature and the middle graph of males from 30 °C incubation temperature (middle panel); the bottom graph indicates the difference between the landscapes, revealing the effect of incubation temperature within a sex on metabolic activity in the adult brain. Illustrated in least squared means of cytochrome oxidase (CO) activity. Positive peaks indicate nuclei that change more in males from a 32.5 °C incubation temperature, whereas negative peaks indicate nuclei that change more in males from a 30 °C incubation temperature. AH, anterior hypothalamus; VMH, ventromedial hypothalamus; AME, external amygdala; SEP, septum; PP, periventricular nucleus of the preoptic area; POA, preoptic area; NS, nucleus sphericus. Orientation of nuclei in each landscape in the two panels are different to show best the patterns of change.

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 fish (see **Chapter 16, Reproductive Plasticity in Fish: Evolutionary Lability in the Patterning of Neuroendocrine and Behavioral Traits Underlying Divergent Sexual Phenotypes**), and the neural correlates of behavioral differences in these species have as yet received little detailed study. However, studies in fish that display alternate male phenotypes suggest that this will be a productive area of research in reptiles.

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. Alternate reproductive phenotypes within a sex avoid this complication since the groups being compared do not differ in 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 relative plasticity hypothesis of Moore (1991) proposes that fixed differences between alternate

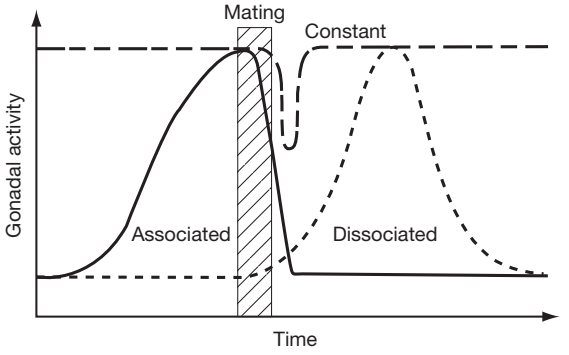

Figure 11 Vertebrates display a variety of reproductive patterns. Here gonadal activity is defined as the development of eggs and sperm and/or increased sex steroid hormone secretion. Individuals exhibiting the associated reproductive pattern (solid line) live in temperate regions where seasonal cycles 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. Note: the dimension of reproductive pattern is depicted as mutually exclusive extremes only for the sake of argument; intermediate forms are known to exist.

phenotypes are due to organizational actions of steroid hormones, while more plastic differences are due to activational influences of these hormones (**Figure 12**). Moore et al. (1998) further refined this hypothesis to account for cases where permanent phenotypic effects might require actions of the relevant hormones only during critical developmental windows. Another model has recently been proposed by Rhen and Crews (2002) that incorporates the Moore model and also other dimensions that include all known alternative reproductive tactics (**Figure 12**).

We review here the two best-characterized models of alternate reproductive phenotype variation from a physiological perspective: the red-sided garter snake (*T. sirtalis parietalis*) and the tree lizard (*Urosaurus ornatus*). We also consider a genus of iguanid lizards, *Sceloporus*, in which variation in male phenotypes is common both within and across closely related species. There are a number of other reptiles displaying alternate reproductive phenotypes whose behavior and ecology are becoming well understood, whereas the endocrinology and neurobiology of behavioral variation has not been studied, such as the side-blotched lizard *Uta stansburiana* (e.g., Sinervo et al., 2000).

23.2.3.1 The red-sided garter snake

The first physiological studies exploring alternate male phenotypes focused on the red-sided garter snake. As described above, this species exhibits a reproductive pattern in which peak levels of gonadal hormones are temporally dissociated from the display of reproductive behavior (Crews, 1991; **Figure 13**). 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). One is an estrogen-dependent attractivity pheromone produced by females that elicits vigorous courtship from males. A 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, which 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 testosterone (Mason and Crews, 1985) and greater abundance of aromatase in the skin (Krohmer, 1989), which presumably converts the endogenous testosterone to estrogen, thereby stimulating production of the female attractiveness pheromone.

23.2.3.2 The tree lizard

The most thoroughly studied lizard that exhibits 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).

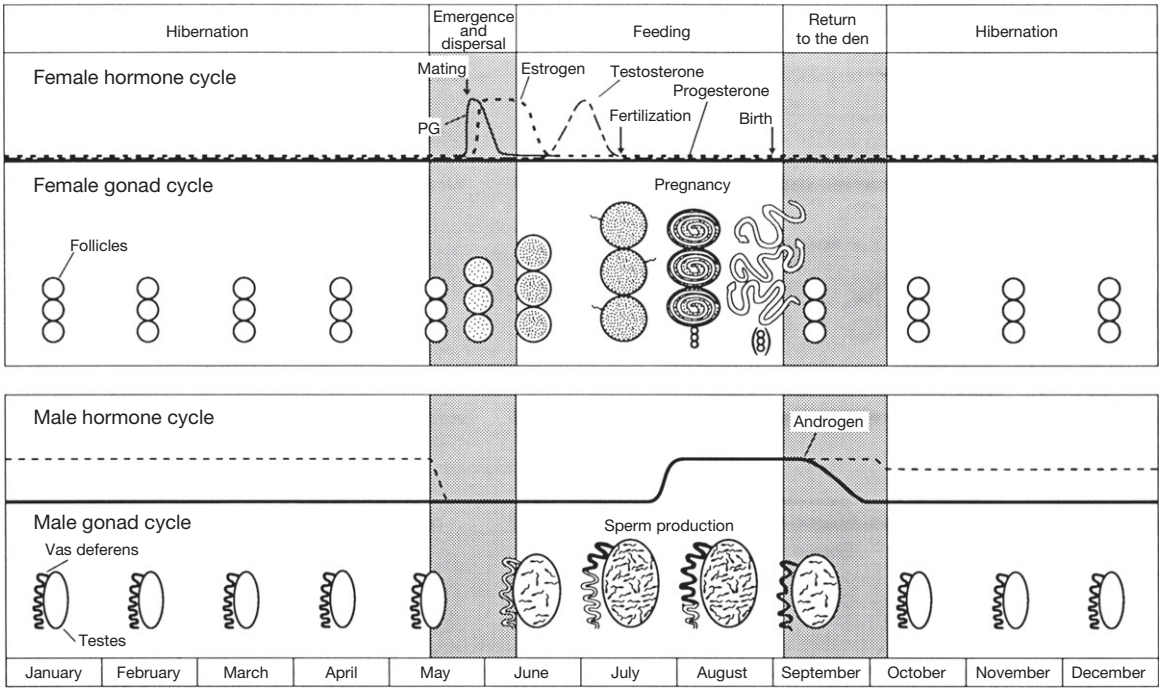

Figure 12 The major physiological and behavioral events in the annual reproductive cycle of the red-sided garter snake in Canada. Animals spend most of the year underground. In the spring, they emerge and mate before dispersing to summer feeding grounds. In the female, mating initiates gonadal growth as well as changes in the hormone profile of the female. Young are born in late summer. Since all metabolic processes slow down during the cold months, androgen levels in the male will be elevated in the spring if he entered hibernation with elevated levels (dotted lines); however, androgen levels usually are basal on emergence (solid line). Sperm are produced during the summer after mating and are stored in the vas deferens (heavy squiggle line next to testis) over winter.

Experiments where males are raised in a common laboratory environment indicate that the basis of this dewlap color variation is either genetic or maternal in origin, since 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) who are site attached and hold territories encompassing the territories of three to four females, versus males with orange dewlaps (orange males) who are more nomadic under poor habitat conditions (i.e., in drought years) and sedentary with small home ranges in good habitat conditions (**Figure 14**). Orange-blue males are more aggressive both in the laboratory and under natural conditions (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 testosterone 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, 1988; Moore and Marler, 1987; Moore et al., 1998). In contrast, castration of neonatal male tree lizards increases the proportion developing as orange males while T implants given on hatching or 30 days thereafter increase the proportions developing as orange-blue males (Hews et al., 1994). By 60 days posthatch, 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., 1998), but much more striking is a clear bimodality in plasma androgen levels on the day of hatch. This possible role for P in determining morph type has experimental support: single injections of P on the day of hatch significantly increased the proportion of males developing

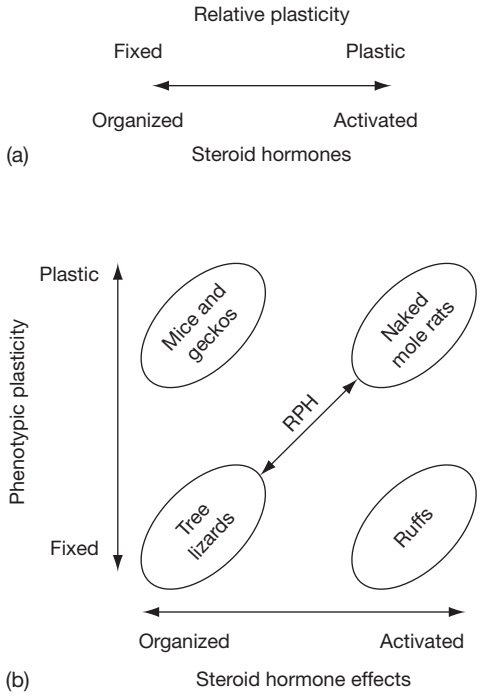

Figure 13 Two hypotheses accounting for alternative reproductive tactics within a sex in vertebrates. (a) Schematic representation of the relative plasticity hypothesis (RPH) of Moore (1991) for the hormonal basis of intrasexual variation in reproductive behavior. (b) Schematic representation of the orthogonal hypothesis of Rhen and Crews (1999) for the hormonal basis of intrasexual variation in reproductive behavior. Here the RPH is a subset of the possible hormonal mechanisms underlying variation in reproductive behavior, but the orthogonal hypothesis also includes species that do not conform to the RPH. Reproduced from Rhen T and Crews D (2002) Variation in reproductive behavior within a sex; neural systems and endocrine activation. *Journal of Neuroendocrinology* 14: 517–532, with permission of Blackwell Publishing.

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 discussed above. More recently, progesterone has been shown to promote an aggressive phenotype in both forms of castrated and implanted morphs (Weiss and Moore, 2004).

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 that 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 testosterone, 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, while changes in space use are not seen in the more aggressive orange-blue males.

23.2.3.3 The side-blotched lizard

The tree lizard work finds support from 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 testosterone, lower year-to-year survivorship, greater endurance, higher activity generally, and occupies larger home ranges that overlap 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 testosterone. 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. T implants can increase home-range size in male side-blotched lizards if not given corticosterone

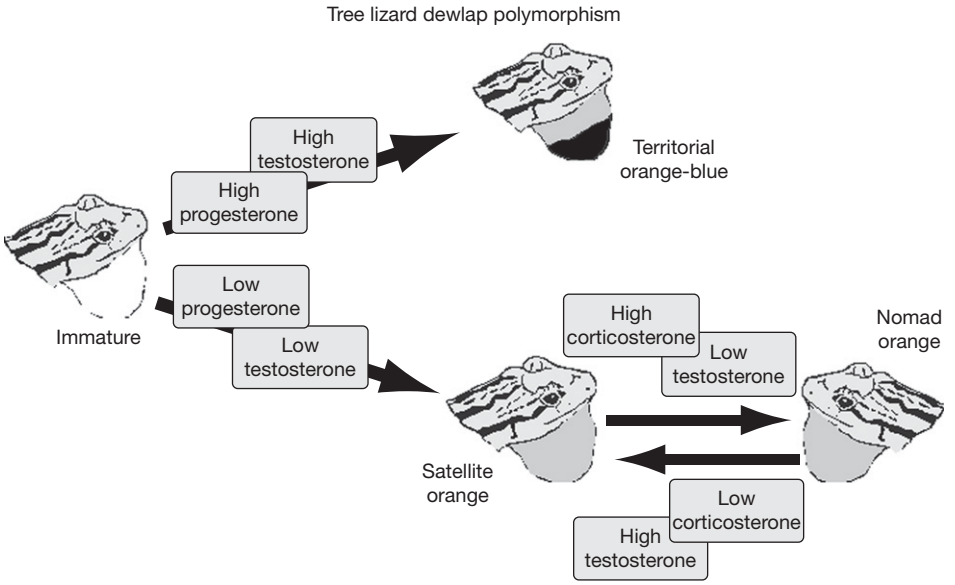

Figure 14 Moore et al. (1998) model for organizational and activational influences on male phenotype development in tree lizards. Both testosterone and progesterone acting during early development affect adult dewlap coloration. Plastic switches between satellite and nomad tactics in adult males of the less aggressive orange morph are hypothesized to be due to an interplay of testosterone and corticosterone. Reproduced from Moore MC, Hews DK, and Knapp R (1998) Hormonal control and evolution of alternative male phenotypes: Generalizations of models for sexual differentiation. *American Zoologist* 38: 133–151.

(Denardo and Sinervo, 1994b). Experimentally elevating T levels in yellow- and blue-throated males to those found in orange-throated males also increase both endurance and access to females in nature (Sinervo et al., 2000).

23.2.3.4 The fence lizard

Although not as well-studied from the standpoint of endocrine physiology, other groups of reptiles present opportunities for exploring the mechanisms underlying behavioral variation within and across species. One particularly promising group is the speciose lizard genus *Sceloporus* or the fence lizards. 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 within and between 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 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 given to hatchling males and females (Abell, 1998).

Androgens affect aggressive behavior in both male and female *Sceloporus*. Moore (1986) found elevated testosterone 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). It is possible that aromatization is important for aggression in mountain spiny lizards, at least in females, since aggressiveness in females is correlated with seasonal elevations in both testosterone 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 testosterone 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 testosterone 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 above for tree lizard and *U. stansburiana* males. Male *S. undulatus* show testosterone 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, while females do not show hormonal responses to either male or female encounters.

23.2.4 Parthenogenetic Lizards

In four families of lizard (agamid, gekkonid, teiid, 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 come from *C. inornatus* (Densmore et al., 1989; Conant and Collins, 1998; **Figure 15**).

Parthenogenesis in *C. uniparens* is accompanied by a fascinating reproductive adaptation: females display male-like pseudocopulatory behavior which is indistinguishable from the male-typical courtship and mounting behavior seen in males of their direct sexual ancestor, *C. inornatus* (Crews, 2005; Crews and Fitzgerald, 1980; **Figure 16**). 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., 1986). Indeed, the process is fundamental to reproduction in all living organisms, including various forms in which males do not exist (Crews, 1996b). The display of pseudosexual behavior is associated predominantly with the postovulatory phase of the ovarian cycle when P levels are elevated (Moore et al., 1985a,b). The P sensitivity of male-like

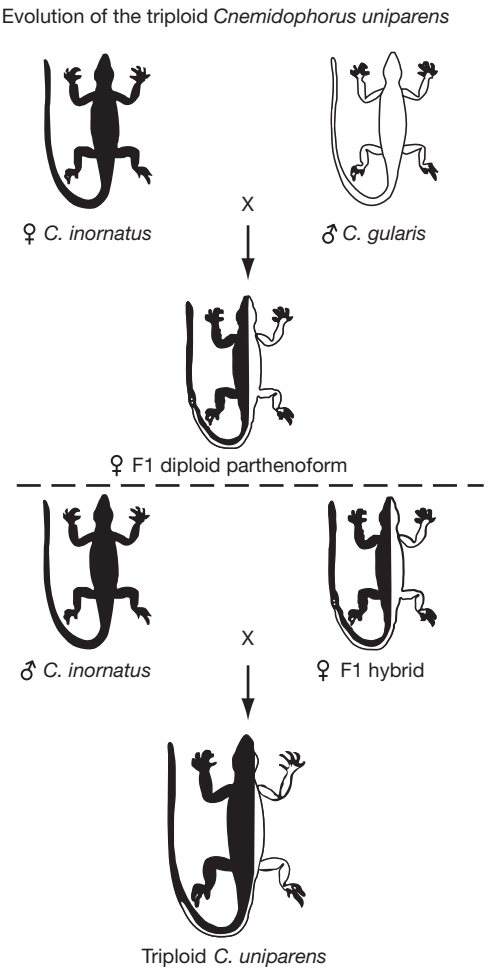

Figure 15 Most whiptail lizard species (genus *Cnemidophorus*) are gonochoristic, having both male and female individuals that reproduce sexually. However, one-third of the 45 species of whiptail lizards are unisexual, consisting only of individuals that reproduce by true parthenogenesis. The parthenogenetic 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*. Reproduced from Godwin J and Crews D (2002) Hormones, brain and behavior in reptiles. In: Pfaff D, Etgen A, Arnold A, Fahrbach S and Rubin R (eds) *Hormones, Brain, and Behavior*, 1st edn. pp 545–585. San Diego, CA: Academic Press, with permission of Elsevier.

pseudosexual behavior in *C. uniparens* has an evolutionary antecedent in *C. inornatus*, where progesterone acting alone and in synergism with androgens stimulates male-typical mounting and intromission

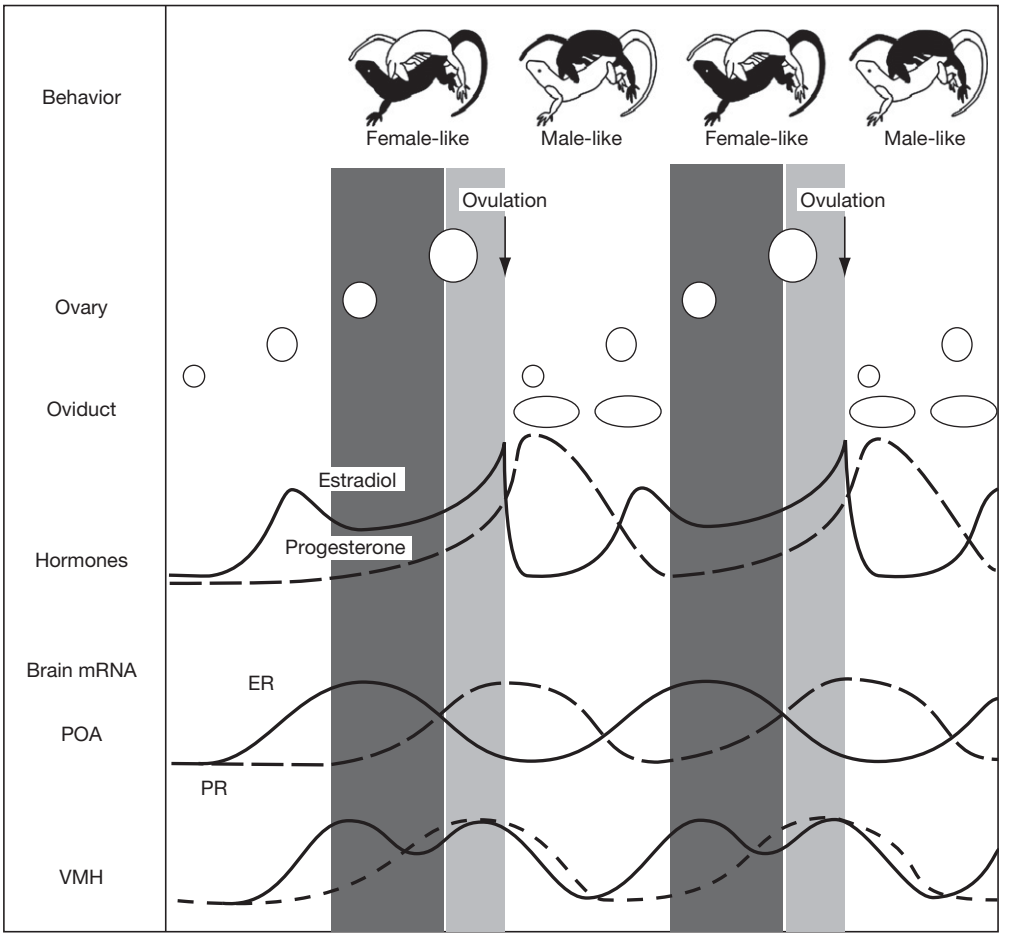

Figure 16 Relationships among male-like and female-like pseudosexual behavior, ovarian state, and circulating levels of estradiol and progesterone during different stages of the reproductive cycle of the parthenogenetic whiptail lizard. The transition from receptive to mounting behavior occurs at the time of ovulation (arrow). Also shown are the changes in abundance of the gene transcripts coding for estrogen receptor (ER) and progesterone receptor (PR) in the preoptic area (POA) and the ventromedial hypothalamus (VMH), brain areas that are involved in the regulation of male- and female-like pseudosexual behaviors. Reproduced from Crews D (1998) The evolutionary antecedents of love. *Psychoneuroendocrinology* **23**, 751–764.

behavior (Lindzey and Crews, 1992). This discovery of P sensitivity in male lizards led to a revisitation of the question and demonstration of similar sensitivity in male rats (Witt et al., 1995). Studies aimed at elucidating the mechanisms by which progesterone is able to activate this normally androgen-dependent behavior are reviewed in the final section of this chapter.

23.2.5 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 cover 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. Recent 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.

23.2.5.1 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 and follicle-stimulating hormone rise during the breeding period in female green sea turtles (*Chelonia mydas*; Licht et al., 1979, 1980). Progesterone and testosterone also rise during this period, although estradiol-17β (E2) does not significantly. Patterns in the loggerhead sea turtle (*Caretta caretta*) show similarities where progesterone, testosterone, 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 et al. (1998) approached this problem by sampling from captive Kemp's Ridley sea turtles (*Lepidochelys kempi*) under seminatural conditions. Male Kemp's Ridley turtles show that testosterone 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 testosterone, E2, and progesterone at the time of mating. Both testosterone and E2 decline sharply after mating, while progesterone declines more slowly.

Social interactions also influence circulating steroid levels in green sea turtles during the mating period. Jessop et al. (1999b) 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., 1999a). Males near, or actually mounting, females have elevated androgen levels, whereas males that are the recipient 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 steroids 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*), where there is some evidence of both photoperiod and androgen control of sexual behavior (Mendonça, 1987a,b).

23.2.5.2 Crocodilians, tuataras, 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 yet 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; Thorbjarnarson and Hernandez, 1993; Tucker et al., 1998).

Data on tuataras, limited to a single extant species representing the order Sphenodontida, are similarly limited. Female tuataras 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, being low during the winter, rising in the spring, and peaking in midsummer to early autumn during the mating period. Female tuataras show elevated levels of estradiol and testosterone during vitellogenesis, which fall at ovulation, when progesterone levels rise. Females also show elevations in plasma arginine vasotocin (AVT) during oviposition relative to during the nest digging and guarding stages that are likely associated with oviductal 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.

23.3 Neuroanatomical Substrates of Sexual Behavior in Reptiles

The neural pathways subserving sexual behaviors in reptiles appear to involve a network of limbic nuclei that, in many cases, have clear homologs in the more complex nervous systems of birds and mammals. The final common pathway for male-typical mounting and intromission behavior appears to be the preoptic area–anterior hypothalamus (POAH), while that of female-typical receptive behavior is the ventromedial portion of the hypothalamus (VMH; comparable to the ventromedial nucleus of the hypothalamus of rodents), as is the case in rodents (DeVries and Simerly, 2002). Both of these areas are rich in steroid hormone receptors, and their activity and neurochemistry respond to both steroid hormones and environmental signals. Several kinds of studies have been performed to establish the roles of various brain areas in controlling sexual behaviors, including the critical importance of the POAH in regulating male-typical behavior and VMH in regulating female-typical suites of reproductive behaviors. Examples of five such types of experiment are described below.

23.3.1 Hormone Receptor Expression

An obvious property of a brain area involved in the control of a hormone-influenced behavior is that it ought to express receptors for that hormone. Receptor expression has been studied in reptiles by several methods, including autoradiography with radiolabeled ligands, immunohistochemistry, and *in situ* hybridization. Accumulation of tritium-labeled E2, testosterone, and DHT is found at a variety of sites in the brain of green anole lizards (Morrell et al., 1979). Halpern et al. (1982) performed a similar study with labeled E2 and testosterone in the brain of red-sided garter snakes. These studies, similar to those in mammals and other vertebrates (Morrell and Pfaff, 1978), reveal substantial E2 binding in the POA, AH, amygdaloid nuclei, and ventromedial and posterior hypothalamic nuclei with lighter labeling in several areas including the septum, torus semicircularis, central gray, and some brainstem areas. In the green anole binding of tritiated testosterone and DHT are very similar to patterns for tritiated E2, but there are some differences in pallial and the mesencephalic tegmental area.

More recently, 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 (reviewed in Crews, 2005; Young and Crews, 1994). 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, sex and species differences in the hormonal regulation, developmental influences on the sexual differentiation of hormonal responsiveness, as well as documenting social influences on ERα and PR mRNA abundance in hypothalamic nuclei. Regulation of receptor expression is discussed in a later section, and the discussion that follows is limited to the neuroanatomical distribution of hormone receptors.

As in other groups of vertebrates, reptiles show what Pfaff et al. (1994) termed a lawfulness of steroid receptor distribution in the brain. Indeed, this conservation in distribution provides evidence of neural homologies as with the expression of AR in the lizard DVR and the avian magnocellular nucleus of the anterior neostriatum (MAN; see below). 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). A number of genes involved in both sex determination and sexual differentiation of brain and behavior of reptiles have been identified and studied. The resulting clones were used to generate riboprobes for use in *in situ* hybridization. Such studies have documented expression of ER and AR 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.

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 dorsal, posterior, and VMH (**Figure 17**). 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 E2 and testosterone, respectively, and it is possible that some of the T label was aromatized to E2 (see Wade (1997, 2005)) and then bound to the ER. This is discussed at greater length below.

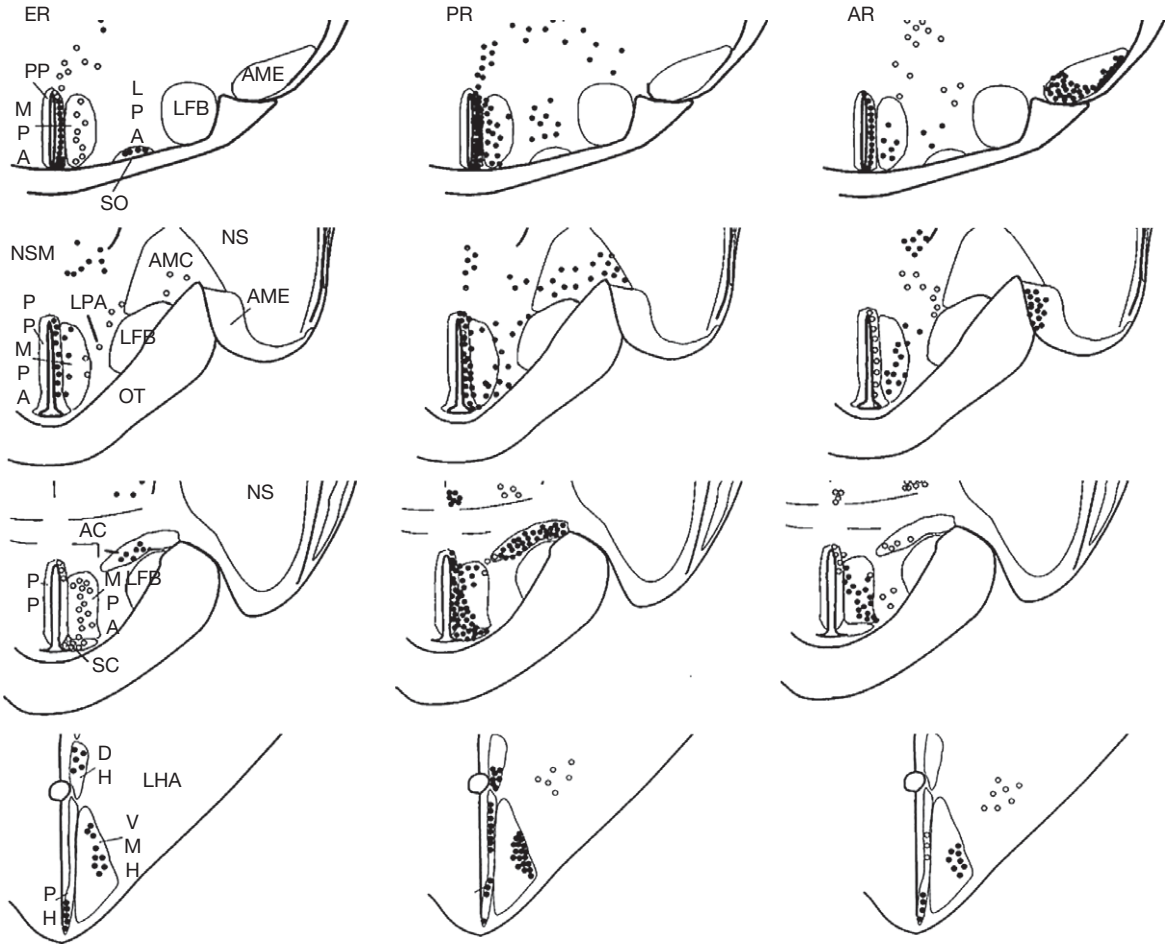

Figure 17 Distribution of cells expressing steroid receptor mRNA in selected sections of the brain of whiptail lizards. Shown are the positions of cells expressing mRNA for estrogen receptor (ER, column 1), progesterone receptor (PR, column 2), and androgen receptor (AR, column 3) in the right half of brain sections. Solid circles indicate heavily labeled cells and hollow circles indicate lightly labeled cells. AC, anterior commissure; AMC, nucleus centralis amygdalae; AME, nucleus externus amygdalae; DH, nucleus dorsalis hypothalami; LFB, lateral forebrain bundle; LHA, lateral hypothalamic area; LPA, lateral preoptic area; MPA, medial preoptic area; NS, nucleus sphericus; NSM, nucleus septalis medialis; PH, nucleus periventricularis hypothalami; PP, nucleus periventricularis preopticus; SC, nucleus suprachiasmaticus; SO, nucleus supraopticus; VMH, nucleus ventromedialis hypothalami. Redrawn from Young LJ, Lopreato GF, Horan K, and Crews D (1994) Cloning and *in situ* hybridization analysis of estrogen-receptor, progesterone-receptor, and androgen-receptor expression in the brain of whiptail lizards (*Cnemidophorus uniparens* and *C. inornatus*). *Journal of Comparative Neurology* 347: 288–300.

PR mRNA expression is also widely distributed through the brain of whiptail lizards (**Figure 17**). Especially strong expression is seen in the VMH and both the periventricular and medial POA as well. Strong labeling is also found in the medial septum, lateral POA, central amygdala, AH, the posterior, dorsal, and VMH, the lentiformis thalamis pars plicta, and the torus semicircularis. Lower levels of PR mRNA are evident in the lateral hypothalamic area, near the premammillary nucleus, and in parts of the optic tectum.

In both the whiptail lizard and the leopard gecko, AR mRNA expression occurs in the DVR, external nucleus of the amygdala, mPOA, AH, LS, dorsolateral anterior nucleus, VMH, periventricular nuclei of the hypothalamus, and especially the premammillary nucleus (Young et al., 1994; Rhen and Crews, 2000). This distribution reveals differences from previously documented distributions in the brain of green anole lizards and garter snakes based on steroid autoradiography. Specifically, AR mRNA is more

abundant than ER mRNA in the LS, 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 DVR of the lizard. The DVR appears homologous to the AR-expressing 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 through *in situ* hybridization has also been employed more recently in green anole lizards to examine the distribution of AR mRNA expression (Rosen et al., 2002). AR mRNA was found in many of the same areas expressing this message in the brain of whiptail lizards with some differences. The regions of similarity include the POA, septum, amygdala, striatum, premammillary nucleus, VMH, and torus semicircularis and brainstem motor nuclei. In contrast to the patterns observed in whiptail lizards, AR mRNA expression is not localized to the DVR of green anole lizards in this study.

Immunocytochemical studies, such as those of Moga et al. (2000), have 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 androgen receptor-immunoreceptivity (AR-ir) in the fence lizard, *S. undulatus*. This method allows distinguishing 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 described above. 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 (BNST). These investigators also found both nuclear staining in the mPOA, periventricular hypothalamus, and ventromedial, premammillary, and arcuate nuclei. AR-ir-fiber staining occurred throughout the AH and POAs and in a variety of other diencephalic areas. Females showed nuclear AR-ir in fewer areas than males, with nuclear staining only in the ventral posterior amygdala and VMH (the ventral posterior 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 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 mPOA than females (Godwin et al., 2000), just as females show higher levels of estrogen-induced PR mRNA expression in the VMH (Crews et al., 2004; Wennstrom et al., 2003). The results reported for fence lizards could be very similar if females do express AR mRNA in the mPOA, but AR protein at levels too low to be detected by immunocytochemistry. A difference in sensitivity of the technique could also account for the lack of AR-ir observed in the dorsal lateral thalamic nucleus and anterior dorsal ventricular ridge, a result that also contrasts with the described distribution of AR mRNA in whiptail lizards (Young et al., 1994).

Thus, *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 by the availability of antibodies that bind specifically to target proteins in reptile tissue. Advances in this area enabling immunocytochemical work exploring co-localization of different receptor types within cells would be particularly useful.

23.3.2 Intracranial Hormone Implants

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 role of these brain areas implicated in hormone-dependent behaviors can be tested by intracranial implantation of minute amounts of steroid hormones directly into the candidate region of animals deprived of systemic sex steroids by gonadectomy. This approach has been shown to effectively restore male-typical sexual behaviors in rats and other mammals (reviewed in DeVries and Simerly (2002)). 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 will induce copulatory behaviors in both castrated male whiptails (*C. inornatus* (Rozendaal and Crews, 1989) and the parthenogenetic *C. uniparens* (Mayo and Crews, 1987; **Figure 18**). Intracranial implantation of progesterone 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).

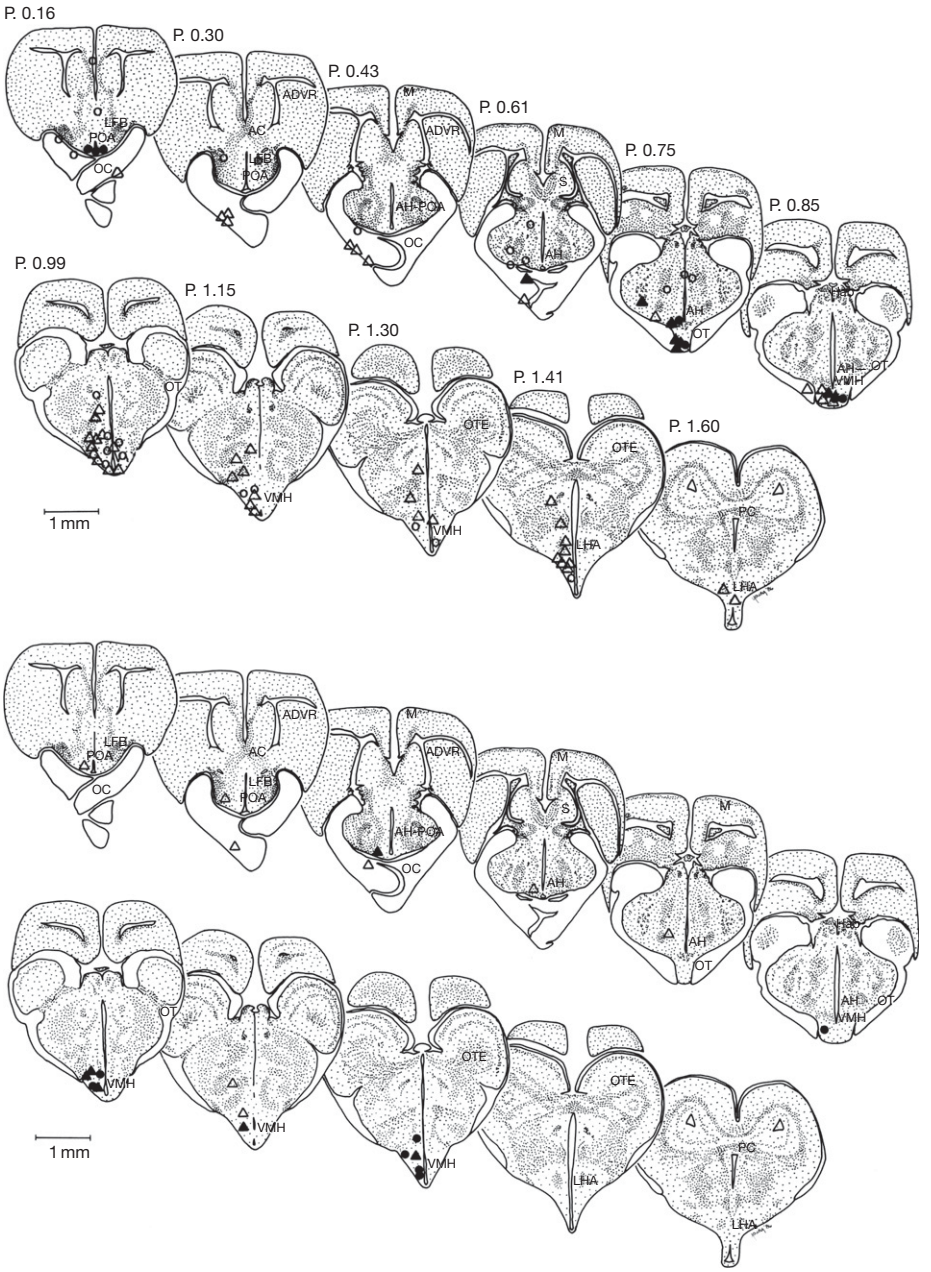

Figure 18 Frontal sections through the brain of a representative whiptail lizard, showing locations of the approximate center of hormone implants that elicited mounting and copulatory behavior (upper panel) or sexual receptivity (lower panel) in whiptail lizards. Numerals indicate distance posterior to zero point. In the upper panel, 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. In the lower panel, solid triangles indicate locations of estrogen implants that resulted in female-like sexual receptivity in ovariectomized parthenogenetic whiptails (*C. 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 to respond. AC, anterior commissure; ADVR, anterior dorsal ventricular ridge; AH, anterior hypothalamus; LHA, lateral hypothalamic area; LFB, lateral forebrain bundle; OC, optic chiasm; OT, optic tract; OTE, optic tectum; PC, posterior commissure; POA, preoptic area; VMH, ventromedial hypothalamus. Reproduced from Godwin J and Crews D (2002) Hormones, brain and behavior in reptiles. In: Pfaff D, Etgen A, Arnold A, Fahrbach S and Rubin R (eds) *Hormones, Brain, and Behavior*, 1st edn. pp 545–585. San Diego, CA: Academic Press, with permission of Elsevier.

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

23.3.3 Morphological Parameters

Another simple prediction is that a brain area involved in the control of a behavior will be influenced structurally by parameters known to affect that behavior. An area involved in mediating a seasonal behavior would be expected to change seasonally, an area involved in a sexually dimorphic behavior ought to exhibit sexual dimorphism in structure, and so on. 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, while females have a larger VMH (Crews et al., 1990). These sexual dimorphisms in size are under the control of gonadal androgens in the male (**Figure 19**). That is, castration of breeding animals results in a reduction in the area of the POA, and an enlargement in the area of the VMH, whereas androgen replacement therapy reverses these effects of castration (Wade et al., 1993). 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 within these areas. Male *C. inornatus* have larger soma sizes in the POA, while 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, while useful, measurements of brain nucleus volume and soma size likely do not reflect many important differences in function. We return to this topic below in considerations of metabolic capacity, neurotransmitter function, and regulation of steroid hormone receptor expression.

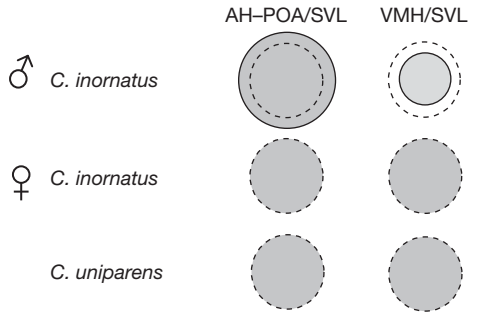

Figure 19 Schematic representations of the volumes of the sexually dimorphic areas in the brain relative to body size in sexual and parthenogenetic whiptails. To aid in comparison, the volume of the anterior hypothalamus–preoptic area (AH–POA) and the ventromedial nucleus of the hypothalamus (VMH) of female *Cnemidophorus inornatus* is represented as a bold outline in other drawings to indicate significant differences. SVL, snout vent length. Reproduced from Godwin J and Crews D (2002) Hormones, brain and behavior in reptiles. In: Pfaff D, Etgen A, Arnold A, Fahrbach S and Rubin R (eds) *Hormones, Brain, and Behavior*, 1st edn. pp 545–585. San Diego, CA: Academic Press, with permission of Elsevier.

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 **Chapter 25, Neuroendocrine Regulation of Reproductive Behavior in Birds** and **Chapter 26, Neural and Hormonal Control of Birdsong**). 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).

More recently, information has become available on sexual dimorphisms in both the brainstem 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 pushups, 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 females (Wade, 1998). Motoneurons 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 in females (O'Bryant and Wade, 1999). There is, however, no consistent relationship between either the breeding/nonbreeding season or androgen treatment (T propionate) and these characteristics in males. T implantation into juvenile females 30-days post-hatching increases cartilage length and size of the dewlap muscle fibers compared to sham-implanted controls (Lovern et al., 2004), suggesting that testosterone exposure during development serves to masculinize components of the neuromuscular system. Another study found that, unlike some other vertebrate systems where the sexes differ in their display behavior, green anoles show no sex difference in the dendritic arborization of motoneurons in AmbX or AmbIX/VIImv (O'Bryant and Wade, 2000). 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 (2000) propose that this may be due to the fact that, while extension of the dewlap is associated with male courtship, females will also lower the hyoid, thereby exposing the patch of red. Taking advantage of the natural variation in copulatory behavior (studs vs. duds), it has been reported that dewlap extension is positively correlated with size of the associated muscle as well as soma size in the amygdale (Neal and Wade, 2007). The same study also suggests that androgen-sensitive tissues in studs might be more responsive to testosterone than those in duds, potentially explaining some of the behavioral variation.

In the variantly colored tree lizard, *U. ornatus*, males show different levels of territorial behavior and have been used to model aggression and mating tactics in socially relevant settings. Research indicates that males on average possess a larger POA, amygdala, and ventromedial nucleus of the hypothalamus than females (Kabelik et al., 2006), with the same study reporting changes in limbic nuclei volume across the breeding season. It is noteworthy that the authors conclude that the observed alterations in brain nuclei volume might not be involved in the regulation of aggressive interactions but that they might play a role in reproductive behavior.

The research with whiptail, green anole, and tree lizards described above focused on species with the familiar pattern of genotypic sex determination. The other end of the genotype–environment spectrum is represented by species that exhibit TSD. In the leopard gecko (*E. macularius*), both low and high incubation temperatures produce females, while intermediate temperatures result in male determination and differentiation (**Figure 3**). Interestingly, sexuality covaries with incubation temperature somewhat independently of gonadal sex, such that females produced at higher temperatures are masculinized relative 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 et al. (1997) found that females from male-biased incubation temperatures had larger POA volumes than those from female-biased incubation temperatures. Indeed, the variation within each sex across temperature morphs is greater than the variation between the sexes from each incubation temperatures. 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 results contrast with those found in females (Crews et al., 1997). Young males show larger volumes for the POA and VMH than do older males, but females do not show these differences with age.

23.3.4 Metabolic Indicators of Neural Activity

The involvement of a particular brain area in a particular behavior is associated with an increase in metabolic activity in the area during expression of the behavior. The 2-deoxyglucose (2-DG) technique enables postmortem assessment of recent metabolic

activity in the brain, and can be used to compare groups of animals engaged in different behaviors. The technique utilizes the dependence of neurons on glucose as a source of energy, and ^{14}C-radiolabeled 2-DG, analog of glucose that can be taken up by cells but not metabolized. The labeled 2-DG accumulates in cells, and this accumulation can be assessed by the relative amounts of radioactivity present in tissue sections (Cada et al., 1995). 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 (**Figure 20**). Specifically, animals displaying male-like pseudocopulatory behavior showed a sixfold greater accumulation of 2-fluoro-2-DG in the mPOA 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.

The results from *C. uniparens* are paralleled across seasons in red-sided garter snakes. Male red-sided garter snakes that actively court females show 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, even simply being exposed to females increases overall 2-DG accumulation in male garter snakes. These results suggest both a generalized arousal effect as well as a more specific effect of active courting, which 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). Upon emergence from hibernation, females initially are receptive to male-courtship behavior but become unreceptive immediately following mating. Females that are courted and then mated have significantly higher activity in the POA and significantly lower activity in the VMH compared to females who are courted but not mated (**Figure 21**). Since intromission during mating is responsible for the loss of sexual receptivity in the female (Mendonça and Crews, 1990; Mendonça et al., 2003; Ross and Crews, 1977; Whittier and Crews, 1989; Whittier et al., 1985, 1987), injection of a local anesthetic (tetracaine or lidocaine)

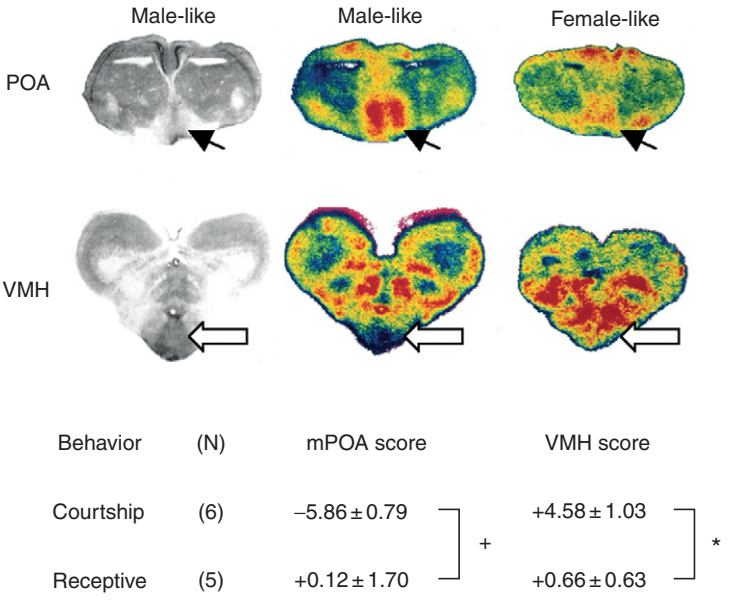

Figure 20 Metabolic activity during pseudosexual behavior in the unisexual lizard. Depicted is average change in 2-deoxyglucose (2-DG) uptake relative to whole brain/optic tract (WB/OT) in the brain of two individual lizards engaged in a pseudocopulation. Left column indicates light micrographs of brain section at the level of the POA (top row, solid arrow) and the VMH (bottom row, open arrow). Other columns are pseudocolor images where red denotes maximum accumulation of 2-DG and green the lowest accumulation. Middle column is the brain of the individual exhibiting male-like pseudosexual behavior while the right column is the brain of the lizard exhibiting female-like pseudosexual behavior. Bottom indicates differences in accumulation at each brain area. Adapted from Rand MS and Crews D (1994) The bisexual brain – sex behavior differences and sex differences in parthenogenetic and sexual lizards. *Brain Research* 663: 163–167.

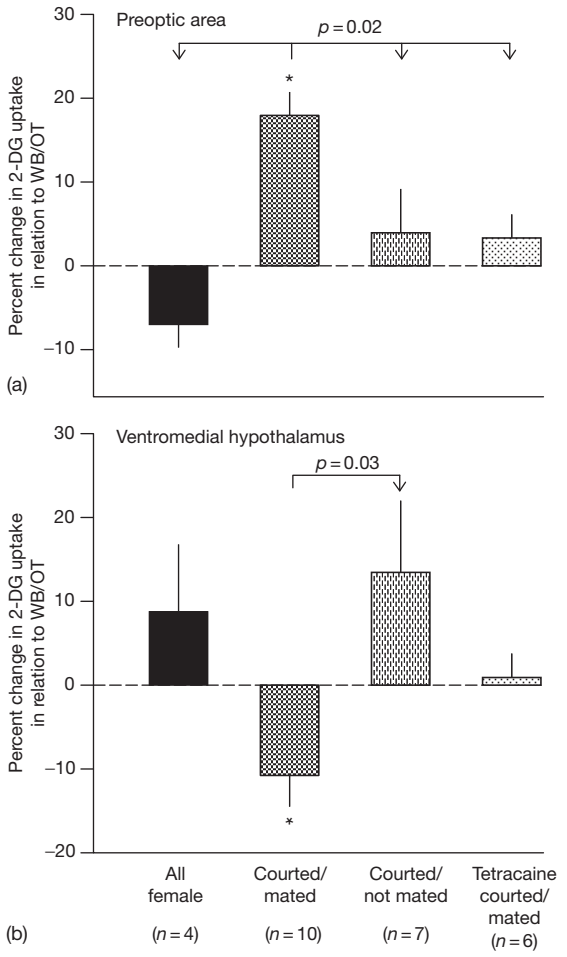

Figure 21 Effect of mating on metabolic activity in the brain of female red-sided garter snakes (*Thamnophis sirtalis parietalis*). Depicted is the relative change in 2-deoxyglucose (2-DG) uptake in different treatment groups relative to whole brain/optic tract (WB/OT). Note that tetracaine, a local anesthetic applied to the cloaca, abolishes the response to mating. The dashed line indicates background brain levels (e.g., zero difference from background). A positive change represents higher accumulation, a negative change lower accumulation than background. Asterisks represent significant differences with level of the difference is indicated. (a) Depicts uptake of 2-DG in the preoptic area/optic tract (POA/OT). (b) Depicts uptake of 2-DG in the ventromedial hypothalamus/optic tract (VMH/OT). Redrawn from Mendonça MT, Daniels D, Faro C, and Crews D (2003) Receptivity and 2-deoxyglucose uptake in female red-sided garter snakes. *Behavioral Neuroscience* 117: 144–149, with permission of the American Physiological Association.

into the cloacal region desensitizes the female to mating stimuli (Mendonça and Crews, 1990, 2001). Not only will this treatment prevent the mating-induced surge in estrogen levels in the plasma and subsequent ovarian recrudescence, but also 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.

23.3.5 Focal Lesions

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 (*A. 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 ER were effective (**Figure 22**). 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.

23.4 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, but some progress has been made in understanding the neurochemical mechanisms underlying agonistic behavior in *Anolis* lizards, and copulatory behavior in whiptails.

23.4.1 Agonistic Behavior in Anoles

Propper et al. (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, amygdala area, DVR, striatum, nucleus accumbens, septum, VMH, LH, medial forebrain bundle, median eminence, pars nervosa, nucleus of the

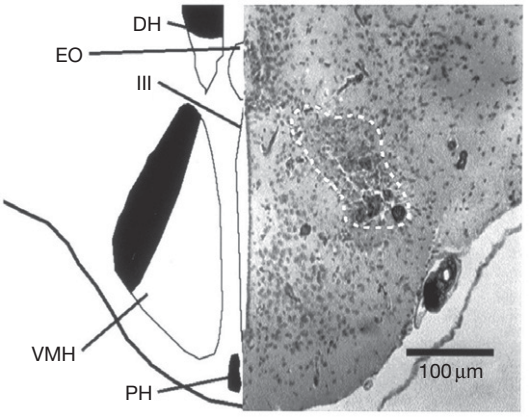

Figure 22 Composite illustration of the ventral portion of the hypothalamus of *Cnemidophorus uniparens* at the level of the ventromedial nucleus of the hypothalamus (VMH). The left side of the figure shows the outlines of the VMH, dorsal hypothalamus (DH), the ependymal organ (EO), the third ventricle (III), and the periventricular hypothalamus (PH). The black regions represent the location of estrogen receptor mRNA . The right side of the figure is a 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 effective prevented estrogen induction of sexual receptivity. Adapted from Kendrick AM, Rand MS, and Crews D (1995) Electrolytic lesions to the ventromedial hypothalamus abolish receptivity in female whiptail lizards, *Cnemidophorus uniparens*. *Brain Research* 680: 226–228.

solitary tract, locus ceruleus, cerebellar cortex (granular layer), dorsal part of the nucleus of the lateral lemniscus, substantia nigra, and myelencephalon. There is generally a 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- 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-like immunoreactivity also occurs in female green anoles, where females with large preovulatory follicles have higher AVT concentrations in the supraoptic area than 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 those 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 to 5-hydroxytryptamine (5-HIAA/5-HT) ratios and the substrate for 5-HT, 5-hydroxytryptophan (5-HTP) (indicating enhancement of both serotonin turnover and production) 1 h after an encounter with a dominant individual (Summers and Greenberg, 1995). The difference between subordinates and both dominants and control males diminishes thereafter. Dominant males show broadly similar patterns, but return to baseline turnover levels more rapidly. Serotonergic signaling via the 5-HT2C receptor is thought to play a role in the dominance observed as part of such agonistic interactions (Baxter et al., 2001). Neither the dopaminergic nor adrenergic systems showed similar patterns, indicating that 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 h 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 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 (DA), epinephrine, and norepinephrine relative 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 relative to males whose eyespots were darkened (suggesting an aggressive or dominant opponent). Feeding green anoles with crickets injected with L-DOPA increases dopaminergic activity

(without affecting the serotonergic and noradrenergic systems) in the medial amygdala and hippocampus coupled with a decrease in aggressive behavior (Hoglund et al., 2005). The authors suggest that this result might be due to D2 receptor signaling in the indirect basal ganglia system of *A. carolinensis*. The involvement of dopaminergic activity in aggressive behavior is corroborated by the observation that lizards viewing an opponent with artificially darkened eyespots exhibit decreased aggression, assume a subordinate status with elevated DA levels measured in the medial amygdala, lateral amygdala, and raphe (Korzan et al., 2006). Conversely, anoles that view an opponent with hidden eyespots express the dominant phenotype with increased aggression, and increased DA levels in the striatum, nucleus accumbens, and substantia nigra/ventral tegmental area. Increases in DA levels in different brain nuclei in subordinate as well as dominant animals suggest differential brain systems involved in phenotypic variation observed in variation.

Patterns of monoamine metabolism in mountain spiny lizards (*S. jarrovi*) are similar to those in anoles, where higher serotonergic activity is seen in nonterritorial satellite (subordinate) males relative to territorial males (Matter et al., 1998). As with anoles, aggressive defense of territory in *S. jarrovi* males results in increases in both 5-HTP and 5-HIAA/5-HT ratios. These interactions also increase activity of the central DA 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 min after males received low-dose systemic injections of corticosterone (1.6–2.0 mg kg^{-1}), but not following tenfold higher corticosterone doses (16–20 mg kg^{-1}; Summers et al., 2000). T injections (1.6–2.0 mg kg^{-1}) enhanced serotonin turnover in the hippocampus, but not in 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 may be part of a mechanism enabling an individual to respond to changing social situations. Plasma corticosterone levels are elevated in animals exposed to a video of a dominant male compared to an animal shown a nonsocial video (Yang and Wilczynski, 2003). Repeated exposure of these lizards to the video of a dominant male elicits a decrease in aggressive behavior that is not observed in animals administered metyrapone (an inhibitor of corticosterone synthesis). Consequently, it has been postulated that the increase in corticosterone levels after an aggressive encounter might serve to habituate an animal's subsequent response (decreased aggression) by influencing memory of the social interaction.

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 DA activity in the telencephalon than did subordinate females, while subordinate females showed higher serotonergic activity in the brainstem. It was suggested that the heightened serotonergic activity in dominant females is more directly related to interactions with males than those with other females.

23.4.2 Copulatory Behavior in Whiptails

23.4.2.1 Dopamine

DA is also integral to the display of copulatory behaviors in male mammals and birds. Woolley et al. (2001) showed that a DA 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. 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 above), the triploid parthenogen is more sensitive, indicating that the parthenogen may have elevated levels of D1 receptor in those limbic brain areas modulating courtship behavior. Not only does this work extend to reptiles and 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. Measurements of the size and number of tyrosine hydroxylase-immunoreactive cells (TH-ir) across the reproductive cycle in females of the both species reveal that in the PvPOA the somal size of TH-ir cells is larger in *C. uniparens* (Woolley et al., 2001; **Figure 23**). Further, while there is no change in the number of TH-ir cells across the reproductive cycle in the ancestral species, in the parthenogen there are fewer cells prior to ovulation, when the individual is exhibiting receptive behavior, compared to the post-ovulatory phase, when individuals are displaying pseudocopulatory behavior (Woolley and Crews, 2004; Woolley et al., 2004a,b; **Figure 16**). Somal area is an important determinant of connectivity and related to the extent of the dendritic arbor (Szaro et al., 1987) and the size of the neuronal receptive field (DeVries and Simerly, 2002). Increases in the size or number of neurons can increase the amount of synaptic input to a particular nucleus (Rakic, 1975; Szaro and Tompkins, 1987; Tompkins et al., 1984). Thus, species differences in both the size and number of cells in limbic and midbrain nuclei may have dramatic functional consequences for both neural organization and behavior.

23.4.2.2 Serotonin

Serotonergic neurotransmission is involved in gating male- as well as female-like pseudosexual behavior expressed by the parthenogenetic whiptail *C. uniparens*. Increasing serotonin levels in the POA of T-implanted *C. uniparens* via systemic administration of a combination of the monoamine oxidase inhibitor, clorgyline, and the precursor of serotonin, 5-HTP, suppresses male-like pseudocopulation (Dias and Crews, 2006; **Figure 24**). In contrast, administration of pCPA (a tryptophan hydroxylase inhibitor) decreases serotonin levels in the POA as well as increasing male-like mounting behavior.

Serotonin injected into the POA of ovariectomized, T-implanted lizards expressing male-like pseudocopulation suppresses male-like pseudosexual behavior (Dias and Crews, 2008). In a similar experiment, female-like receptivity exhibited by ovariectomized, estradiol-injected *C. uniparens* was suppressed by injection of serotonin into the VMN (Dias and Crews, 2008). Pharmacological targeting of specific serotonin receptors indicates that the 5-HT_{1A} and 5-HT_{2A} receptors are involved in male-like pseudocopulation and female-like receptivity, respectively. *In situ* hybridization used to measure 5-HT_{1A} and

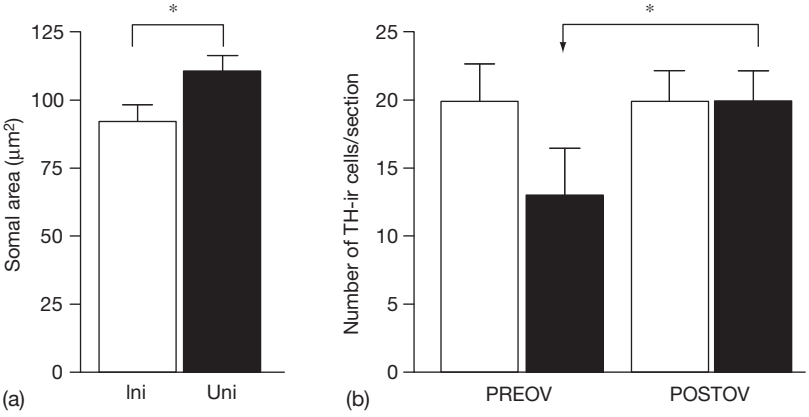

Figure 23 Neuron size and abundance of tyrosine hydroxylase in the periventricular preoptic area of female whiptail lizards. (a) Tyrosine hydroxylase immunoreactive (TH-ir) cells in *Cnemidophorus. uniparens* (uni, black bars) are larger than those of female *C. inornatus* (ini, open bars), presumably reflecting ploidy. (b) However, there is a greater number of TH-ir cells in the PvPOA of the descendant parthenogenetic species during the postovulatory ovarian stage (POSTOV), when male-like pseudocopulatory behavior is being exhibited, compared to the preovulatory stage (PREOV); females of the ancestral sexual species show no change in cell number. Mean ± SEM shown. Adapted from Wooley SC, Sakata JT, and Crews D (2004b) Tyrosine hydroxylase expression is affected by sexual vigor and social environment in male *Cnenidophorus inornatus*. *Journal of Comparative Neurology* 476: 429–439.

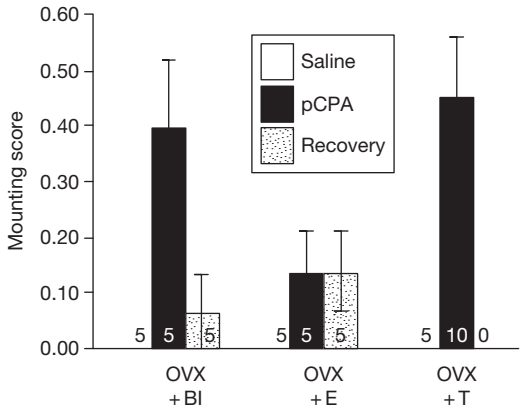

Figure 24 Relationship between steroid hormones, serotonin manipulation, and male-like pseudosexual behavior in the unisexual whiptail lizard, *Cnemidophorus uniparens*. pCPA, a tryptophan hydroxylase inhibitor that reduces serotonin biosynthesis, induces male-like mounting in ovariectomized lizards receiving a blank (Bl) or testosterone (T) treatment, but not ovariectomized lizards receiving estrogen (E). Animals were tested after receiving four daily injections of saline, four daily injections of pCPA beginning 2 days after the saline behavioral tests, and 2 weeks (recovery). These data suggest that E prevents the pCPA induction of mounting behavior. Data from Dias B and Crews D (2006) Serotonergic modulation of male-like pseudocopulatory behavior in the parthenogenetic whiptail lizard, *Cnemidophorus uniparens*. *Hormones and Behavior* 50: 401–409.

5-HT_{2A} receptor mRNA levels indicates that testosterone and estradiol regulate mRNA levels of these receptor subtypes in the POA and VMN.

More recent studies have measured serotonin and receptor mRNA levels in naturally cycling animals. These results closely parallel the data generated in the hormonally manipulated conditions. It appears that the hormonal regulation of serotonin and receptor subtype mRNA levels at the POA and VMN and subsequent signaling via these receptors at these brain nuclei allows for the reciprocal inhibition that characterizes behavior associated with either hormonal state (e.g., the facilitation of male-like pseudocopulation in androgen-implanted animals, while female-like receptivity is simultaneously suppressed).

23.4.2.3 Nitric oxide

Nitric oxide (NO) is produced in neurons from arginine by the enzyme nitric oxide synthase (NOS) and is thought to play a critical role in both peripheral and central control of reproductive behavior (Nelson et al., 1997). In male rats it is involved in the control of male copulatory behavior and is influenced by testosterone (Du and Hull, 1999), while in female rats it has been shown to influence P-mediated lordosis behavior (Mani et al., 1994). This responsiveness to the two different steroids and involvement in copulatory behavior suggested a possible involvement in the neural control of pseudocopulation in *C. uniparens* which display male-like pseudosexual behavior under the influence of progesterone normally, but can be made to display the same behavior with T treatment. The role of NO in this behavior was investigated by treatment with the arginine analog *N* nitro-*l*-arginine methyl ester (L-NAME), which inhibits NOS. Using a repeated-measures design, ovariectomized hormone-treated lizards that were displaying robust pseudocopulatory behavior were treated with an intraperitoneal injection of 100 mg kg^{-1} L-NAME in physiological saline 1 h before testing with a receptive stimulus animal with the same dose of the inactive isomer D-NAME (Sanderson et al., 2005). Following L-NAME-administration latencies to approach, mount and pseudocopulate were increased, and half of the individuals failed to pseudocopulate at all, while the behavior in D-NAME-treated animals was similar to baseline.

Another candidate in addition to DA for the locus at which progesterone might engage the cellular pathway normally activated by androgens is NO. Since both neurotransmitters are involved in T-dependent male-typical copulatory behavior, and independently in P-dependent female-typical behavior, ectopic expression of PR in the POA of the parthenogen brain might enable progesterone to activate male-like pseudocopulatory behavior via one or both of these molecules. Pharmacological blockade of NOS suppresses pseudocopulatory behavior in whiptails (Sanderson et al., 2005, 2006) as it does in rats. Preoptic NOS is upregulated by T exposure sufficient to activate male-like copulatory behavior in female whiptails (Sanderson et al., 2006) and in males (Sanderson et al., 2008), and also at the time of the ovarian cycle when male-like copulatory behavior is displayed. Furthermore, analysis of citrulline production suggests that NO is synthesized more in the POAs of whiptails displaying T-dependent male-typical copulatory behavior than in controls deprived of sex steroids (and therefore not copulating). The molecular mechanisms of NOS upregulation have been studied in some detail, using laser capture microdissection and quantitative PCR (qPCR) to examine the time course of NOS induction following T administration to gonadectomized whiptails. For example, if castrated male whiptails implanted with testosterone are tested at four different time points,

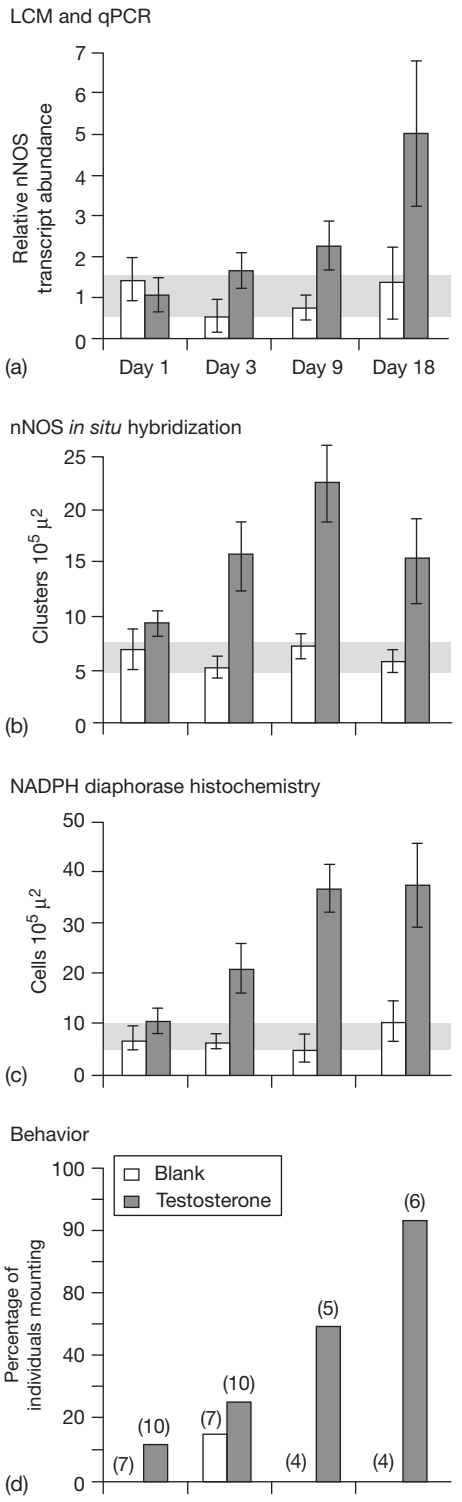

Figure 25 The effect of androgen on behavior, neurochemistry, and gene expression in the male whiptail lizard (*Cnemidophorus inornatus*). (a) Percentage of castrated males exhibiting male-typical copulatory behavior after various lengths of time following implantation of testosterone-filled (gray bars) or blank (empty bars) Silastic capsules. (b) Expression of nNOS in the periventricular preoptic area (PPA) as revealed by *in situ hybridization*. Presented are the numbers of nNOS-expressing cells inferred from silver grain clusters in the PPA. For comparison, the 95% confidence interval around the global mean of the blank-treated animals is shown as a light gray band. (c) Relative nNOS transcript abundance in laser-microdissected fragments of PPA. Raw measures of nNOS transcript abundance are expressed relative to 18S ribosomal RNA abundance and normalized to the mean value of the blank-implanted individuals. (d) Expression of nitric oxide synthase as inferred from NADPH diaphorase histochemistry. Presented are numbers of NADPHd + cells in the PPA. For comparison, the 95% confidence interval around the global mean of the blank-treated animals is shown as a light gray band. Numbers in parentheses above bars are group sizes. Modified from Sanderson NSR, Le BD, Zhou Z, and Crews D (2008) Preoptic neuronal nitric oxide synthase induction by testosterone is consistent with a role in gating male copulatory behaviour. *European Journal of Neuroscience* 27: 183–190.

their sexual behavior, nNOS transcript abundance, and parallel NADPH diaphorase histochemistry indicate that nNOS is upregulated by testosterone in a pattern consistent with a role in mediating hormonal gating of copulatory behavior (**Figure 25**).

23.4.2.4 *Arginine vasotocin*

AVT and its mammalian homolog vasopressin are involved in a variety of male-typical behaviors, including aggression and courtship (see **Chapter 22, Sensorimotor Processing Model: Neuroendocrine Control of Reproductive Behaviors in an Amphibian**). It has been recently reported that T administration increases the number of AVT-ir cells in the POA of female *C. inornatus* (Hillsman et al., 2007; **Figure 26**). In keeping with a facilitatory role for AVT in male-courtship behavior of the roughskin newt (Moore and Miller, 1983; Moore et al., 1981), and sexually dimorphic populations of AVT-ir cells in a variety of species (males > females), Hillsman et al. (2007) hypothesize that androgenic modulation of AVT immunoreactivity might be central to the expression of male sexual behavior in the whiptail lizards studied.

23.5 Regulation of Sex Steroid Hormone Receptors

The ability to localize and compare relative abundance levels of the three main gonadal steroid hormone receptors in whiptail lizards has allowed

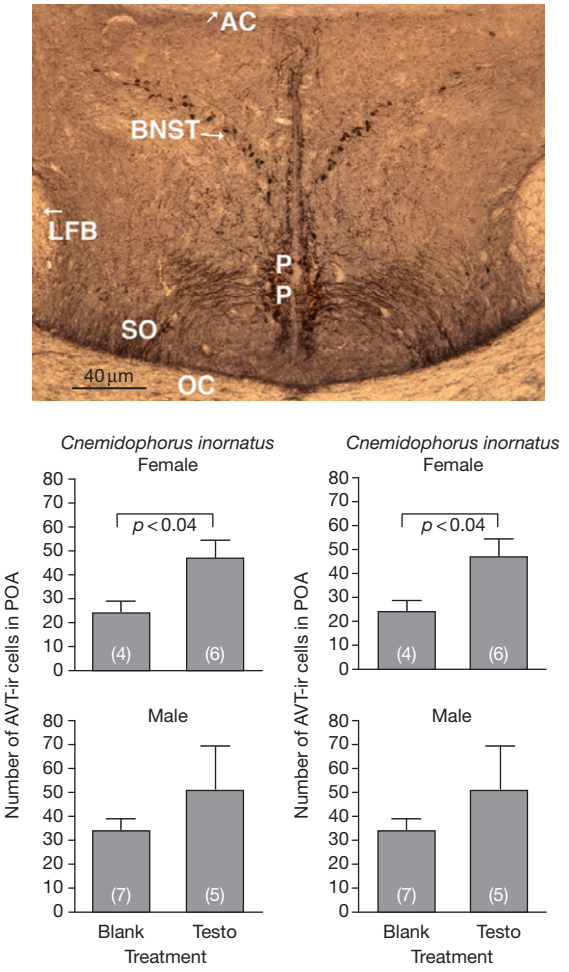

Figure 26 Arginine vasotocin immunoreactive cells (AVT-ir) and fibers in the preoptic area (POA) and the bed nucleus of the stria terminalis (BNST) in a Virago *Cnemidophorus uniparens* (aromatase inhibitor-created males) receiving a blank implant (top panel). AC,anterior commissure; LFB, lateral forebrain bundle; OC, optic chiasm; SO, supraoptic nucleus; PP, periventricular preoptic area. Bottom left panel: testosterone treatment increases abundance of AVT-ir in the POA of *Cnemidophorus inornatus*. Effect size is large in both females (top) and males (bottom), but reaches statistical significance only in females. Bottom right panel: testosterone treatment increases abundance of AVT-ir in the POA of *Cnemidophorus uniparens* treated as embryos with aromatase inhibitor (Viragos) (bottom), although not in *C. uniparens* treated as embryos with ethanol (Parthenoform) (top). Mean ± SEM shown with number of individuals in parentheses. Redrawn from Hillsman KD, Sanderson NS, and Crews D (2007) Testosterone stimulates mounting behavior and arginine vasotocin expression in the brain of both sexual and unisexual whiptail lizards. *Sexual Development* 1: 77–84.

a variety of questions related to sex and species differences in their regulation to be addressed. Estradiol increases ER mRNA abundance in discrete brain regions in the whiptail lizards. Young et al. (1995a) documented this using a 0.5 µg injection of estradiol benzoate (EB) and measuring ER mRNA abundance 24 h after administration. The EB effectively stimulates female-typical receptive behavior in parthenogenetic whiptail lizards as well as increases ER mRNA in some regions (torus semicircularis and VMH), decreases it in others (LS), 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 two 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 to that seen in rats where estrogen downregulates its receptor. This difference between whiptail lizards and rats may relate to differences in the nature of their ovarian cycles. Whiptail lizards have elevated E2 levels for a relatively long period prior to ovulation and display receptive behavior for the duration of this period, while female rats are receptive for only a short window following ovulation. Young and Crews (1995) suggest that prolongation of the length of time E2 levels is elevated and of sexual receptivity may be quite common in mammals (e.g., cats and rabbits). Lastly, 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 (**Figure 27**). That is, an inverse relationship exists between 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 E2 that, in turn, results in lower levels of circulating E2 through feedback effects.

Estradiol 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

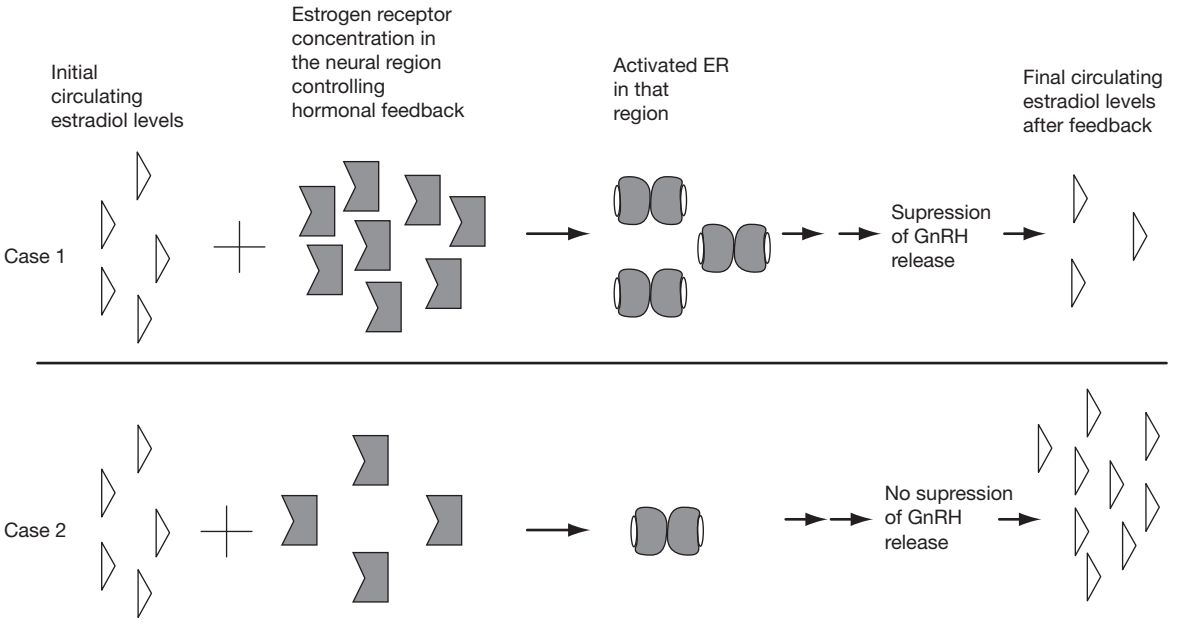

Figure 27 Schematic illustrating the sensitivity compensation model for species differences in the circulating concentrations of sex steroid hormones. Two different situations (= species) are illustrated that differ in the abundance of estrogen receptor (ER) in the neurons involved in the negative feedback 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 receptor, 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. Redrawn from Young LJ and Crews D (1995) Comparative neuroendocrinology of steroid receptor gene expression and regulation: Relationship to physiology and behavior. *Trends in Endocrinology and Metabolism* 6: 317–323.

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* (Young et al., 1995b), with EB being more effective in the parthenogenetic *C. uniparens* (**Figure 28**). EB also effectively stimulates increases in PR mRNA in the POA of female *C. inornatus*, again with similar dosages being more effective in the parthenogen *C. uniparens* than 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 (Godwin et al., 1996; **Figure 29**).

While E2 increases PR mRNA in both the VMH and POA of female and parthenogenetic whiptail lizards, exogenous progesterone inhibits both female-typical receptive behavior and decreases estrogen-stimulated ER- and PR mRNA in the VMH (Godwin et al., 1996). This effect of progesterone on both receptivity and ER and PR mRNA abundance is similar to patterns in well-studied rodent models (see **Chapter 2, Feminine Reproductive Behavior and Physiology in Rodents: Integration of Hormonal, Behavioral, and Environmental Influences**). In contrast, exogenous progesterone 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 (see **Chapter 2, Feminine Reproductive Behavior and Physiology in Rodents: Integration of Hormonal, Behavioral, and Environmental Influences**). 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 testosterone, however,

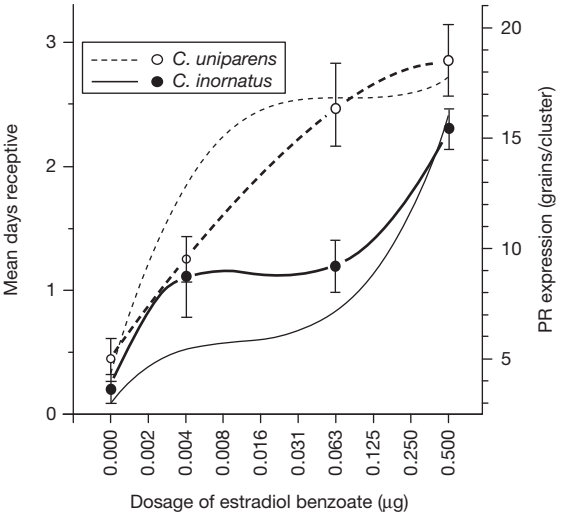

Figure 28 Species differences in neuroendocrine controlling mechanism. Species differences in the induction of sexual receptivity (thin lines) and progesterone receptor mRNA expression (thick lines) by estradiol benzoate (EB) in ovariectomized whiptail lizards. Ovariectomized animals were given a single injection of EB and either tested daily for receptivity for 4 days following the injection or brains were removed 24 h after treatment and analyzed using *in situ* hybridization. Vertical error bars represent standard errors of the mean. Reproduced from Young LJ, Crews D (1995) Comparative neuroendocrinology of steroid receptor gene expression and regulation: Relationship to physiology and behavior. *Trends in Endocrinology and Metabolism* 6: 317–323, Copyright (1995) Elsevier.

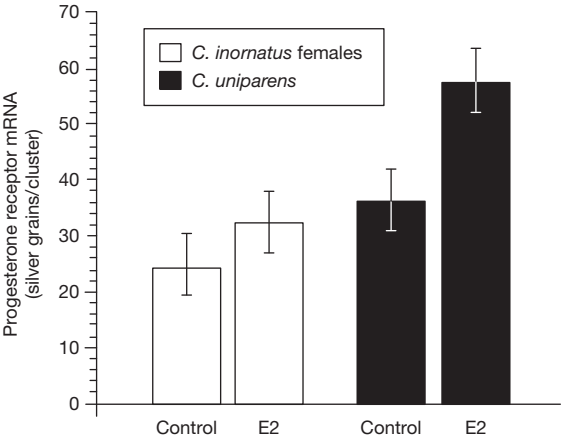

Figure 29 Evolution of a novel neuroendocrine controlling mechanism. Progesterone receptor mRNA levels in the PvPOA for female *C. inornatus* (open bars) and *C. uniparens* (black bars) given either blank or estradiol injections. Depicted is the abundance of progesterone receptor mRNA measured as average number of silver grains per cluster (mean ± SEM) in the periventricular region of the preoptic area of the ancestral sexual (*C. inornatus*) and descendant parthenogenetic (*C. uniparens*) whiptail lizards. Adapted from Godwin J and Crews D (1999) Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: Regional and species differences. *Journal of Neurobiology* 39: 287–293.

did not show an attenuation of the female pattern of responsiveness. These results indicate that maintenance of the male-typical pattern of nonresponsiveness requires the activational effects of testosterone while 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 progesterone 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 progesterone (directed at the POA) than P-insensitive males (Crews et al., 1996b). Interestingly, there are also differences in both PR- and AR mRNA abundance between the two groups following intracranial implantation of progesterone. P-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 mPOA, external amygdala, and LS. No differences are seen between P-sensitive and P-insensitive males without an intracranial implant.

Sex and species differences are also found in androgenic regulation of ER, PR, and AR mRNA. Implantation of gonadectomized male and female *C. inornatus* and parthenogen female *C. uniparens* with either testosterone 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. Also there are species effects in that the triploid parthenogen shows higher steroid receptor mRNA abundances overall than the diploid sexual females. Finally, aromatization of testosterone to estrogen is likely important in some regions. PR mRNA in the periventricular POA is increased in both males and females by testosterone, but not by nonaromatizable DHT.

Lastly, individual experiences might influence gene expression in the brain directly rather than via modulation of the endocrine physiology of the partner. For example, in the hamster and the rat, exposure to sexual behavior of the opposite sex induces expression of the immediate-early gene *c-fos* in those brain regions that mediate sexual behavior. 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 of reptiles and other vertebrates, patterns of steroid receptor regulation vary greatly. 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 reproductive cycles. 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 co-localization of receptor types within neurons and crosstalk between signaling 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.

23.6 Conclusions and Future Directions

Reptiles enable 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 that great diversity exists among vertebrates in reproductive behaviors and the neuroendocrine mechanisms underlying these behaviors. For example, study of species with dissociated reproductive tactics and unisexual species has suggested three factors which 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 350 million years ago, yet research in reptiles has revealed apparent conservation of many behavioral controlling mechanisms between these groups. For example, research with reptiles has led us to re-examine certain assumptions in behavioral neuroendocrinology. One such example concerns the idea that progesterone is a female-specific hormone with no function in males. Experiments with four lizard species have 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, 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 (males) and ovaries (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 animal finds 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 the question now becomes why the activation of one cascade (e.g., the ovary-determining cascade) actively suppresses the complementary sex-determining cascade. This understanding, and the obvious fact that many reptiles lack sex chromosomes, requires that the construction of a new paradigm to take the place of the organized–default

concept, as generalizing this canonical concept to all vertebrates appears debatable. Species with environmental sex-determining mechanisms are a case in point (Crews, 1993). In those with temperature-dependent sex determination, gonadal sex is determined by incubation temperature during the middle of development. In hermaphroditic species, sex change occurs in the adult as a result of changes in the social environment.

What can replace the heterogamety hypothesis and still account for the evidence at hand (**Figure 30**)? There can be little doubt that the original vertebrate was a female and that males evolved only after the evolution of self-replicating (= female) organisms. Males have been gained (or lost), but females remain. Considering the female as the fundamental sex and the male as the derived sex would account for all of the above observations. It also suggests the intriguing possibility that males may be more like females than females are like males. In rodents, the relative ease of masculinizing individuals compared to the difficulty in defeminizing individuals suggests this to be the case. Thus, George Bernard Shaw may have posed the wrong question when he asked, "why can't a woman be more like a man?" A better and more interesting question may in fact be "why might males be more like females, rather than females like males?"

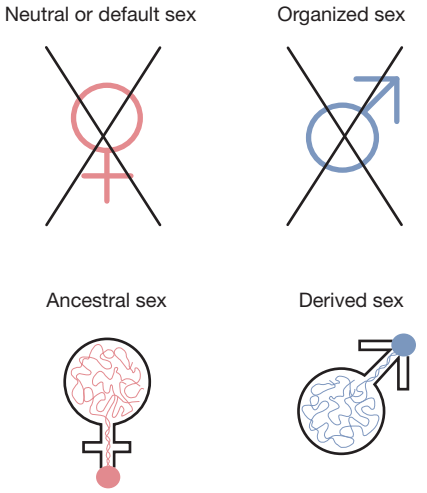

Figure 30 Canonical and alternative concepts of sexual differentiation. The top portion depicts the classic organization concept as it is used to describe the sexual differentiation process. The female is regarded as the neutral or the default sex, whereas the male is regarded as the organized sex. This model emphasizes the differences between the sexes. However, research indicates that his paradigm does not apply to organisms lacking sex chromosomes, nor does it satisfactorily explain the accumulated data on animals with sex chromosomes. The bottom portion depicts a suggested paradigm that combines the sequence in the evolution of sex and a change in emphasis. There is no doubt that what we call a female was the original sex and hence can be regarded as the ancestral sex, whereas males arose after females are derived from them. This view recognizes that both sexes are organized states and raises the possibility that males may be therefore like females than females are like males, and emphasizes the similarities between the sexes as well as their differences. Modified from Crews D (1993) The organizational concept and vertebrates without sex chromosomes. *Brain, Behavior and Evolution* 42: 202–214.

The mechanisms that generate individual variation are an important focus across modern biology. Understanding these mechanisms is of fundamental importance for understanding a broad range of phenomena, from the very basic question of how evolutionary change takes place, to the very 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. Evidence of environmental influences on the organization of behavior was first obtained in studies of nonmammalian vertebrates that lack sex chromosomes; it was proposed that the neural organization underlying sex-typical behaviors depends upon behavioral or physical stimuli in the environment (Crews, 1994). Sex-changing fish typify the former, in which case the social environment effectively switches the brain and behavior, and ultimately the gonad, from one sex to the other (Godwin and Crews, 1999; Godwin et al., 2000). The latter instance is characteristic of reptiles that depend upon the temperature of the incubating egg in the midtrimester of embryonic development to determine gonadal sex.

An example of the evolution of genetic control of sexual behavior 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 heterogametic (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 (Y) trigger for male development is absent,

the male-determining cascade can be activated by treating embryos with aromatase inhibitor, producing fully functional males (Wennstrom and Crews, 1995; Wibbels and Crews, 1994; Hillsman et al., 2007). Such animals exhibit only male-like copulatory behavior. 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 appears in whiptail lizards not only to influence brain anatomy but also to suppress 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. While this gives insight into these mechanisms in this the most speciose group of reptiles, little is still 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 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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Biographical Sketch

David Crews is Ashbel Smith Professor of zoology and psychology at the University of Texas at Austin. He received his PhD in psychobiology in 1973 under the supervision of the late Daniel S. Lehrman and Jay S. Rosenblatt from the Institute of Animal Behavior of Rutgers University. After a postdoctoral fellowship in biochemical endocrinology at the University of California at Berkeley, he moved to Harvard University in 1975 first as an assistant, then associate professor of biology and psychology. He moved to the University of Texas at Austin in 1982. His research primarily concerns sex determination and sexual differentiation; specifically, his research has included studies (1) the mechanisms and outcomes of sex determination in vertebrates lacking sex chromosomes; (2) the evolution of hormone–brain–behavior mechanisms; (3) understanding how the environment and behavior influence the structure and function of the brain; and (4) the role of epigenetics in behavioral neuroendocrinology. Dr. Crews has worked with a wide variety of organisms, from fruit flies to mammals, but focuses on reptiles. He has received various honors, including a Sloan Fellowship in neuroscience, the Distinguished Scientific Award from the American Psychological Association, and a MERIT Award and a 20-year Research Scientist Award from the NIMH. He has been elected fellow of the American Association for the Advancement of Science, American Psychological Society, and the American Academy of Arts and Sciences.

Nicholas Sanderson graduated from Emmanuel College, Cambridge, in 1991, with the degree of Bachelor of Arts. He then worked as an English teacher in association with Summit School of Languages in Mito, Japan, until May 1997, when he entered the Psychology Department of Hiroshima University in Japan, receiving a second Bachelor of Arts degree in 2001. He recently received his PhD in 2008 in neuroscience from the University of Texas at Austin.

Brian Dias received his BS in life sciences and biochemistry from St. Xavier's College (Mumbai, India) in 2000. He then went on to finish his MS in neuroscience in 2003, in Dr. Vidita Vaidya's laboratory at the Tata Institute of Fundamental Research (Mumbai, India). During this time he investigated the molecular and cellular targets of stress and antidepressant treatments in the rat brain. In keeping with Brian's broad interests in animal behavior and physiology, he joined the laboratory of Dr. David Crews at UT-Austin in spring 2004, and since then, has been investigating the neurobiology underlying sexually dimorphic behavioral repertoires, and recently received his PhD in 2008 from the University of Texas at Austin.