2.10  **Hormones, Brain, and Behavior in Reptiles**

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### 2.10.1 Introduction

172

### 2.10.2 Diversity in Sex Determination, Sexual Differentiation, and Hormone—Behavior Relationships

173

#### 2.10.2.1 Temperature-Dependent Sex Determination

173

#### 2.10.2.1.1 Mechanisms of Temperature-Dependent Sex Determination

173

#### 2.10.2.2 Organizing Influence of Incubation Temperature in the Leopard Gecko

175

#### 2.10.2.3 Alternative Mating Strategies and Tactics

177

#### 2.10.2.3.1 The Red-Sided Garter Snake

178

#### 2.10.2.3.2 The Tree Lizard

178

#### 2.10.2.3.3 The Side-Blotched Lizard

180

#### 2.10.2.3.5 Other

186

#### 2.10.2.4 Parthenogenetic Lizards

186

#### 2.10.2.5 Sex Steroids and Behavior: Other Reptiles

187

#### 2.10.2.5.1 Turtles

187

#### 2.10.2.5.2 Crocodilians, Tuatara, and Amphisbaenians

188

### 2.10.3 Neuroanatomical Substrates of Sexual and Aggressive Behaviors in Reptiles

189

#### 2.10.3.1 Hormone Receptor Expression

189

#### 2.10.3.2 Intracranial Hormone Implants

191

#### 2.10.3.3 Morphological Parameters

191

#### 2.10.3.3.1 Sexual Dimorphisms

191

#### 2.10.3.3.2 Seasonal Variation

193

#### 2.10.3.4 Metabolic Indicators of Neural Activity

194

#### 2.10.3.5 Focal Lesions

194

### 2.10.4 Neurochemical Bases of Sexual and Aggressive Behavior in Reptiles

195

#### 2.10.4.1 Nonapeptides

195

#### 2.10.4.2 Monoamines

197

#### 2.10.4.3 Nitric Oxide

201

#### 2.10.4.4 Steroidogenic Enzymes, Cofactors, and Receptors

202

### 2.10.5 Regulation of Sex Steroid Hormone Receptors

203

### 2.10.6 Conclusions and Future Directions

205

### References

206

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**Abbreviations**

- 2-DG 2-deoxyglucose
- 5-HIAA 5-hydroxyindoleacetic acid
- 5-HT 5-hydroxytryptamine (serotonin)
- 5-HTP 5-hydroxytryptophan
- AH anterior hypothalamus
- AmbIX/VII
  - motor nucleus of the facial nerve
- AmbX motor nucleus of the facial nerve
- AR androgen receptor
- AVP avanteroventral periventricular nucleus
- AVT arginine vasotocin
- BNST bed nucleus of the stria terminalis
- CO cytochrome oxidase
- CREB cAMP response element-binding protein
- DA dopamine
- DHT dihydrotestosterone
- DVR dorsoventricular ridge
- E2 estradiol
- EB estradiol benzoate
- EPI epinephrine
- FTP female-producing temperature
- GFP green fluorescent protein
- GSD genotypic sex determination
- HSP hormone-sensitive period
- t-N^G^ nitro arginine methyl ester
- LS lateral septum
- MAN magnocellular nucleus of the anterior nidopallium
- MPT male-producing temperature

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2.10.1 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 (tuatara) (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 purposes of this chapter, discussion will be limited to the familiar ‘reptile’ forms. These animals offer two large advantages from an experimental standpoint. The first advantage is the enormous diversity of patterns of sex determination and sexual differentiation observed across reptilian species, enabling some particularly illuminating ‘natural experiments,’ some of which are discussed below. Among the diverse patterns represented are temperature-dependent sex determination (TSD), mixed genotypic- and temperature-dependent sex-determining mechanisms, reproduction by obligate parthenogenesis (all-female species), and distinct alternate male phenotypes within a species. The second advantage to the study of reptiles is the presence of many phylogenetically primitive characters. Additionally, because mammals and birds arose from turtle-like and crocodilian-like ancestors, respectively, the study of reptiles allows for phylogenetic comparisons to these highly investigated taxa.

This chapter will discuss 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 will focus on both within-sex and between-sex differences in the neurochemistry of brain areas subserving sexual and aggressive behaviors in reptiles. 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 neural 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 control mechanisms in vertebrates. Such comparative research has revealed that the areas in the limbic forebrain involved in the regulation of sexual and aggressive behaviors are ancient and highly 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 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. For instance, the diversity in reptilian characteristics allows for a variety of comparisons between species exhibiting differing patterns of development.

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

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>NS</td>
<td>nucleus sphericus</td>
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<tr>
<td>P</td>
<td>progesterone</td>
</tr>
<tr>
<td>POA</td>
<td>preoptic area</td>
</tr>
<tr>
<td>POAH</td>
<td>preoptic area-anterior hypothalamus</td>
</tr>
<tr>
<td>PostOv</td>
<td>postovulatory</td>
</tr>
<tr>
<td>PR</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>PreOv</td>
<td>preovulatory</td>
</tr>
<tr>
<td>PvPOA</td>
<td>periventricular preoptic area</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative real-time polymerase chain reaction</td>
</tr>
<tr>
<td>siRNA</td>
<td>short interfering RNA</td>
</tr>
<tr>
<td>T</td>
<td>testosterone</td>
</tr>
<tr>
<td>TH-ir</td>
<td>tyrosine hydroxylase immunoreactive</td>
</tr>
<tr>
<td>TSD</td>
<td>temperature-dependent sex determination</td>
</tr>
<tr>
<td>TSP</td>
<td>temperature-sensitive period</td>
</tr>
<tr>
<td>VMH</td>
<td>ventromedial hypothalamus</td>
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and, for many processes there are sufficient numbers of species, including outgroups, to generate adequate sample sizes for comparisons. For example, viviparity has evolved from oviparity perhaps 100 or more times independently in reptiles ( Blackburn, 1999; Guillette, 1991). Meanwhile, the conserved nature of many reptilian characteristics can lead to renewed study in mammals to further our understanding of the neuroendocrine control of mammalian sexual behavior. For example, the discovery that progesterone (P) 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 P and its receptor in the control of sexual behavior in males (discussed below in detail). 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. The study of sexual behaviors has been important for our understanding of behavioral neuroendocrinology generally and an understanding of the ancestral state of neuroendocrine mechanisms controlling these behaviors should help us better understand how they have evolved and function in birds and mammals.

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

Reptiles show an extraordinary diversity in the patterns of sex determination and sexual differentiation. In addition to GSD, many reptiles possess primitive (e.g., TSD) or specialized (e.g., obligate parthenogenesis; 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 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, 1998). 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, GSD species that are responsive to incubation temperature to the extent that individuals with a male genotype may become functional females, 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.

2.10.2.1 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 (all marine turtles and tortoises and many freshwater turtles), and some lizards (various Geckkonid and Agamid species), gonadal sex is established by the temperature experienced by the incubating egg ( Viets et al., 1994; Janzen and Krenz, 2004; 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 XXY and ZWZZ sex-determining systems ( Robert and Thompson, 2001; Shine et al., 2002; Warner and Shine, 2008; Quinn et al., 2007 ). For example, in the Central Bearded Dragon ( Pogona vitticeps ) females are ZW while males are ZZ (female heterogamety) ( Quinn et al., 2007). Incubation at high temperatures can override genotype to create genetic males that are functional females. Fecundity in these ‘sex-reversed’ males is higher than in ZW females incubated at a female-producing temperature ( Holleley et al., 2015 ).

2.10.2.1.1 Mechanisms of Temperature-Dependent Sex Determination

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

Sex steroid hormones are implicated in the process of TSD, and estrogens in particular appear essential in ovary 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 overide 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, testis 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, 1996; Crews et al., 1994, 2006; Wibbels et al., 1991).

The process of TSD is believed to have evolved by the retrograde addition of regulatory elements upstream of established developmental programs (Wilkins, 1995; Graves, 1995). If this model is accurate, the various sex-determining systems can be thought of as one evolutionarily conserved network for both testis and ovary determination regulated by various taxon-specific upstream factors (McLaren, 1998). Support for this hypothesis is found in the conservation of sex-determining genes among taxa (Crews and Bull, 2009); the same genes important in mammalian gonad determination are found in other species and show strong similarities in their temporal expression patterns during gonadogenesis. The developmental decision of ovary versus testis does not flow through a single gene but is instead determined by a ‘parliamentary’ system involving networks or cassettes of genes that have simultaneous inputs to several components of the downstream cascade. Systems with different balances of the inherited and environmental influences could all operate this way (as they could also operate through a single master), merely by varying the inputs to the networks. These cassettes function much akin to a ratchet (Crews and Bull, 2009; Figure 3). In this instance the cassette consists of a functionally interrelated suite of genes that, during the process of development, are progressive in nature, leading to more advanced tissue types that become increasingly distinct one from the other in form and function (i.e., testis vs ovary). It is significant that this process is reversible initially; that is, the process can be redirected during the early, but not later, developmental stages. In the slider turtle this moment of lability corresponds to the overlapping TSP and hormone-sensitive period.

The genes involved in both the core sex-determining cassette and the hormone-sensitive cassette have had their expression patterns analyzed throughout gonadogenesis by whole-mount and sectioned in situ hybridization as well as quantitative real-time polymerase chain reaction (qPCR) in several species. The red-eared slider turtle is the best-studied model (Ramsey and Crews, 2009; Shoemaker and Crews, 2009; Matsumoto and Crews, 2012), but excellent information is available for the snapping turtle (Rhen and Schroeder, 2010). Under female-producing temperature (FPT) or ovary determination, Rspo1 and FoxL2 are modulated differently than under male-producing temperature (MPT), while under MPT or testis determination, Dmrt1, Sox9, Mis, and Sf1 are modulated differently than under FPT (Ramsey and Crews, 2007a,b, 2010; Fleming and Crews, 2001). Wnt4 appears to be regulated at both FPT and MPT. As described below, embryos shifted from FPT to MPT exhibit a rapid activation of Dmrt1, Mis, and Sf1 gene expression, while Sox9 upregulation is delayed. Conversely, shifting embryos from MPT to FPT upregulates Rspo1 soon after the shift, followed by FoxL2 and Wnt4 upregulation during the end of the TSP. Cassette #2 genes (aromatase, AR, ERα, and ERβ) are present throughout gonadal development, but the intensity of expression differs according to incubation temperature; e.g., aromatase is expressed at higher levels at FPT than at MPT. As gonadal development continues, ERα and AR levels are equivalent between MPT and FPT midway through the TSP, but both exhibit a spike in expression at FPT toward the end of TSP (Ramsey and Crews, 2007b).

As mentioned, administration of exogenous estrogen overrides male-producing temperature effect, leading to ovarian development in slider turtles. However, the gonads of estrogen-treated embryos exhibit a slightly different pattern of gene expressions in aromatase, ERα, and ERβ from the gonads incubated at FPT even though the outcomes of bipotential gonads are uniform. At FPT, both aromatase and ERα are upregulated while ERβ is downregulated; in contrast, administration of
E2 causes both ERα and ERβ to be downregulated in the medullary compartment, possibly indicating a negative feedback effect of estrogen on ERα expression while aromatase expression patterns remain the same (Ramsey and Crews, 2007a, b, 2009).

The ability of estrogens and their mimics to override the MPT signal to direct ovarian development in slider embryos suggest the possible interaction between estrogen and core sex-determining gene expressions. Several studies indicate that estrogen has a suppressive effect on the expressions of testis-determining genes (Dmrt1 and Sox9) and an inducible effect on the expression of ovary-determining genes (Rspo1). Estradiol treatment in developing embryos significantly suppresses Dmrt1 mRNA expression in the slider (Ramsey and Crews, 2007b; Murdock and Wibbels, 2006).

Dmrt1 is a key activational node of the sex-determining cassette and, along with Sox9, is a master regulator of the testicular pathway much as FoxI2 as a regulator of the ovarian pathway (Ramsey and Crews, 2009; Shoemaker and Crews, 2009; Matsumoto and Crews, 2012). These functions have been demonstrated in studies in which their expression is over-expressed or knocked down in *in vitro* gonadal explants. For example, studies with the slider turtle have utilized Dmrt1-green fluorescent protein (GFP) overexpression vector at FPT at which Dmrt1 expression was usually suppressed and short interfering RNA (siRNA) at MPT at which Dmrt1 expression usually is activated. In such studies it is essential that the effects of gene manipulation are validated by qPCR to confirm mRNA levels, and GFP fluorescence to confirm protein levels.

### 2.10.2.1.2 Organizing Influence of Incubation Temperature in the Leopard Gecko
#### 2.10.2.1.2.1 Effects on the General Phenotype

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. Since 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 34–35 °C again produces virtually all females (Sakata and Crews, 2004a; Figure 4). Hence, females from eggs incubated at 26 °C are referred to as low-temperature females, whereas those 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 those from a low temperature (Gutzke and Crews, 1988). Similarly, although males are the larger sex, incubation temperature has a marked effect on growth within a sex. Females from a male-biased temperature grow faster and larger than do females from a female-biased temperature and become as large as males from a female-biased temperature (Tousignant and Crews, 1995).

In leopard geckos, as in other TSD reptiles, administration of exogenous estrogen to the incubation 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 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, not gonadal 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 T 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 5). 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 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 (T) 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 T; treatment with E2 decreases submissive behavior in males from a male-biased embryonic temperature compared to males from a female-biased embryonic temperature (Rhen and Crews, 1999; Huang and Crews, 2012; Figure 6). 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. However, if ovariectomized females from low and male-biased temperatures are treated with T, they

**Figure 4** Pattern of temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. The right panel 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 co-vary, 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 (left panel), whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex.
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 brain nucleus volume, a variety of factors influence metabolic capacity in the brains of leopard geckos. These include incubation temperature, age, and sexual experience. Incubation temperature affects CO activity in both females and males, although the effect varies depending on the brain nucleus being considered. Females from a male-biased incubation temperature have increased metabolic capacity of the anterior hypothalamus (AH), external amygdala, dorsolateral hypothalamus, dorsoventricular ridge (DVR), nucleus sphericus (NS), lateral septum (LS), and striatum but do not increase capacity in the posterior hypothalamus or periventricular preoptic area (PvPOA) (Figure 7).

Of particular interest is the finding that young females from a male-biased incubation show greater CO levels in the medial preoptic area (POA) 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

Figure 5  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., 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. J. Comp. Neurol. 380, 409–421; Gutzke, W.H., Crews, D., 1988. Embryonic temperature determines adult sexuality in a reptile. Nature 332, 832–834; Crews, D., unpublished.
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, 2003, 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 8). As indicated above, incubation temperature during embryonic development influences an individual’s aggressive and sexual behaviors in adulthood. For example, an increase in incubation temperature results in an increase in adult aggressivity in both males and females. Correlated with this are increased amounts of CO in the AH and both the septum and POA. Similarly, female-typical sexual behaviors decline with increasing incubation temperature, and the correlations between the VMH and both the DVR and septum were significant only in females. Correlations among preoptic, hypothalamic, and amygdalar areas tend to be distributed across both sexes, suggesting that there may exist shared pathways underlying the expression of male-typical and female-typical behaviors (Crews et al., 2006; Figure 9).
Thus, a variety of factors including gonadal sex, age, sexual experience, and incubation temperature 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.

2.10.2.2 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 functionally associated. This has led to the long-standing assumption that increasing levels of gonadal steroid hormones in the circulation activates mating behavior. That is, that 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 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 homologous. This will only be determined through comparative analysis.

The display of reproductive behavior in reptiles and other vertebrate groups shows one of three basic temporal relationships to gamete production (Figure 10). The most common relationship is termed an associated reproductive pattern. Animals displaying this pattern exhibit sexual behavior when their gonads are actively producing gametes and steroid hormone levels are elevated. Reptilian examples of this pattern are the green anole lizard (Anolis carolinensis), the sea turtles discussed in the next part of this section, and many of the other species discussed in this chapter.

The display of mating behavior may also be temporally uncoupled from gamete production. This is termed a dissociated reproductive pattern. The most thoroughly reptilian example of this pattern remains the red-sided garter snake, Thamnophis sirtalis parietalis (Crews, 1983). This species is discussed at length below.

Figure 9  Between sex and within sex differences in metabolic capacity 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 (those above plane) indicate nuclei that change in relation to baseline brain metabolic rate 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 is the least squared means of cytochrome oxidase (CO) activity. Positive peaks (those above plane) indicate nuclei that change more in males from a 32.5°C incubation temperature, whereas negative peaks (those below plane) 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 is different to show best the patterns of change.
The third possible temporal relationship between gametogenesis 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 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.

### 2.10.2.3 Alternative Mating Strategies and Tactics

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. Historically, research on alternative reproductive phenotypes in reptiles has focused primarily on behavioral and ecological correlates, though new research is helping us understand the neuroendocrine bases of these phenotypes.

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 phenotypes are due to organizational actions of steroid hormones while more plastic differences are due to activational influences of these hormones (Figure 11). 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. An expansion of this model by Rhen and Crews (2002) incorporates other dimensions that include all known alternative reproductive tactics.

Next, we review three well-characterized models of alternate reproductive phenotype variation from a physiological perspective: the red-sided garter snake, the tree lizard (Urosaurus ornatus), and the side-blotched lizard (Uta stansburiana). We also consider a genus of iguanid lizards, Sceloporus, in which variation in male phenotypes is common both within and across closely related species, as well as some examples from other species.
to being at the center of a mating ball. Subsequent studies revealed that as males emerge from winter conditions, they initially may produce the female pheromone (Shine et al., 2001; LeMaster et al., 2008). This, however, proved to be a transient phase. In collecting and holding all males for several days, it has become clear that the initially attractive males revert back to their normal unattractive state, while the she-males remain attractive for days, weeks, and even 1 year later (RT Mason, 22 January 2016, unpublished communication).

The comparison of males with she-males presents the opportunity for much more examination of the neuroendocrine regulation of sexual behaviors. She-males have higher circulating concentrations of T (Mason and Crews, 1985) and greater abundance of aromatase in the skin (Krohmer, 1989), which presumably converts the endogenous T to estrogen, thereby stimulating production of the female attractiveness pheromone. The difference in circulating T between males and she-males may also translate to differences in hormonal regulation of neural function, an idea supported by the finding that preoptic area-anterior hypothalamus (POAH) morphology differs between males, females, and she-males, with some males possessing larger, and males smaller, POAH volumes than females (Krohmer et al., 2011). Neural aromatase activity also shifts from predominance in the olfactory region during spring courtship to the septal-POAH in the fall, suggesting that the aromatization of androgens into estrogens is also involved in the regulation of pheromone detection and reproduction in this species (Krohmer et al., 2010).

Glucocorticoids have recently also been shown to exert effects on red-sided garter snake behavior, causing a shift in preference from courtship to feeding, and thus likely playing a role in the control of seasonal differences in behavior (Lutterschmidt and Maine, 2014). The underlying mechanism likely involves neuropeptide Y (NPY) (Morris and Crews, 1990).

### 2.10.2.3.1 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 12). 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 and T-inhibited attractiveness pheromone produced by females that elicits vigorous courtship from males (Parker and Mason, 2012, 2014). A second pheromone, produced by males, identifies them as male and hence not the object of courtship.

A small proportion of males (termed ‘she-males’) produce the attractiveness pheromone, which characterizes females, appearing to confuse other males in mating aggregations into expending effort attempting to mate with the she-males (Mason and Crews, 1985). It is thought that, in addition, the she-males derive benefits from being surrounded by the mating ball due to attainment of heat generated from the friction of movement by males, and by the reduction of predation risks by crows due

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**Figure 11** Two hypotheses accounting for alternative reproductive tactics within a sex in vertebrates. Panel A: Schematic representation of the Relative Plasticity Hypothesis (RHP) of Moore (1991) for the hormonal basis of intrasexual variation in reproductive behavior. Panel B: Schematic representation of the Orthogonal Hypothesis of Rhen and Crews (1999) for the hormonal basis of intrasexual variation in reproductive behavior. Here the RHP 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 RHP.

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**Figure 12** The tree lizard is the most thoroughly studied lizard species that exhibits alternative mating strategies. In this species, the males possess colored dewlaps that are extended during both aggressive and sexual interactions (Thompson and Moore, 1991a; reviewed in Moore et al., 1998). The color of this dewlap varies among males and shows at least nine geographic variants with one to five variants occurring in any given location (Thompson and Moore, 1991b). Experiments 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 13). Orange-blue males are often more aggressive both in the laboratory and in nature (Thompson and Moore, 1991a, 1992), though
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**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.

**Figure 13** Moore and coworkers 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 T and corticosterone. Redrawn from Moore, M.C., Hews, D.K., Knapp, R., 1998. Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. Am. Zool. 38, 133–151.
not in all studies. Weiss and Moore (2004) and Kabelik et al. (2006) found no aggression differences between morphs; these particular studies were conducted during a prolonged drought, when population densities of all morphs were well below their usual levels, suggesting complex and not fully understood regulation of these behaviors across environments.

The effects of gonadal steroid and glucocorticoid hormones in male tree lizards support the predictions of Moore’s relative plasticity hypothesis. Adult T and corticosterone levels do not differ between orange-blue and orange males and neither castration nor androgen manipulations alter dewlap color expression in adult males (Kabelik et al., 2006; 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 proportion 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 and castration of orange-throated morphs (Weiss and Moore, 2004), and 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 below. More recently, P has been shown to promote an aggressive phenotype in both forms of castrated and implanted morphs (Weiss and Moore, 2004), and this effect is likely independent of androgenic pathways as the P group failed to show the same effects of increased limbic morphology as did an experimental group treated with T (Kabelik et al., 2008).

Corticosteroids play important roles in short-term behavioral responses in male tree lizards, supporting the relative plasticity hypothesis and adding to a growing body of information indicating these steroids are important mediators of behavioral plasticity in this and other species. Corticosterone levels vary in both orange and orange-blue males depending on habitat conditions, being higher in dry years (Moore et al., 1998). In the aggressive orange-blue males, corticosterone levels are temporarily higher in losers of long-term laboratory dominance interactions but show the opposite pattern in winners of short-duration encounters (Knapp and Moore, 1995). In the field, the less aggressive orange males show both less intense aggressive behavior and greater corticosterone elevations in response to an aggressive encounter than do orange-blue males (Knapp and Moore, 1996). Males of the two morphs also differ in their response to exogenous corticosterone. Both morphs show decreases in circulating T, but this decrease is greater in the subordinate orange males. Knapp and Moore 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 (Knapp and Moore, 1997).

The corticosterone response of male tree lizards to predators, however, does not differ between morphs. Thaker et al. (2009a) demonstrated that while both morph types show similar increases in corticosterone to a predator (Sonoran collared lizard, *Crotaphytus nebulius*), the males of the orange morph display more prolonged subsequent antipredator behaviors (i.e., increased hiding duration and flight initiation distance). In another study, Thaker et al. (2009b) demonstrated the causality of corticosterone in increasing antipredator behaviors in both morph types, with corticosterone treatment increasing hiding durations and display frequency while decreasing latency to first display (Figure 14). These studies further suggest that even when circulating corticosterone concentrations across morphs are equivalent, the orange morph is more sensitive to this hormone, suggesting a possible difference in neural glucocorticoid receptor densities in the relevant brain regions. Alternately, this greater sensitivity to glucocorticoids in orange males may be mediated within the periphery, as the orange male morph possesses lower concentrations of corticosterone-binding globulins in the plasma than does the orange-blue morph (Jennings et al., 2000).

Finally, female tree lizards are also polymorphic and evidence is now emerging for differences in mate choice preferences between female morphs (Lattanzio et al., 2014). Although female tree lizards show very low aggression levels, these findings pave the road for further research into the neuroendocrine regulation of sexual behaviors across female tree lizard morphs.

**2.10.2.3.3 The Side-Blotched Lizard**

The tree lizard work finds support from studies in the side-blotched lizard. This species has three male morphs that are distinct in both morphological characteristics and behavior, and these differences are genetically determined (Sinervo and Lively, 1996; Sinervo et al., 2001). 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. These morph differences seem at least partly to be the result of differential regulation of T by gonadotropins in a morph-specific manner (Mills et al., 2008). The orange-throated morph has higher plasma levels of T, 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 terraria (Denardo and Licht, 1993) and both home range size and activity levels in the field (Denardo and Sinervo, 1994a), even when corticosterone is given in combination with T. This effect of corticosterone on male home range appears to depend on interactions with neighboring males (Denardo and Sinervo, 1994b). Corticosterone-implanted males decreased home range size if some neighboring males were saline-implanted, but not if all neighboring males received corticosterone implants. Testosterone implants can increase home range size in male side-blotched lizards if not given with corticosterone (Denardo and...
Sinervo, 1994b). Experimentally elevating T levels in yellow- and blue-throated males to those found in orange-throated males also increases both endurance and access to females in nature (Sinervo et al., 2000).

More recent research on side-blotched lizards has expanded investigation into the realm of the brain, demonstrating that the male morphs differ in relative hippocampal (homolog) volume in relation to territory/home-range size and that rates of neurogenesis within the hippocampus is related to habitat complexity in the territorial morphs (LaDage et al., 2009, 2013).

2.10.2.3.4 Fence Lizards
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 the development of sexual phenotypes 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). A recent comparison of androgen receptor (AR) density in S. undulatus and S. virgatus also revealed that a male-biased sex difference is present in the POA and VMH in the former species while no sex difference exists in the latter (Hews et al., 2012). This latter finding suggests that receptor distribution differences across Sceloporus species and sexes not only underlie differences in body coloration but also in social behavior.

Androgens affect aggressive behavior in both male and female Sceloporus. Moore (1986) found elevated T associated with territorial behavior during the nonbreeding season in male mountain spiny lizards, Sceloporus jarrovi. This territorial aggression is affected by both castration and T replacement (Moore, 1987a,b). 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 T and estradiol (E2) (Woodley and Moore, 1999a), but ovariectomized, T-implanted females lacked some elements of aggressive behavior observed in sham-operated controls (Woodley and Moore, 1999b). Elevated T and the associated territorial behavior have substantial energetic and survival costs in male S. jarrovi that appear to result from increases in energy expenditure without compensating increases in energy intake (Marler and Moore, 1989, 1991; Marler et al., 1995). Comparable energetic and growth costs of experimentally elevated T levels are seen in male northern fence lizards (S. undulatus hyacinthus) (Klukowski et al., 1998). Corticosterone concentrations are also correlated with territory size in S. undulatus, perhaps due to the need to mobilize larger
energy stores to patrol a larger territory; the benefit of that extra energy expenditure seems to be an increase in the opportunity to sire offspring, as offspring number correlates strongly with corticosterone level and territory size (John-Alder et al., 2009).

Sceloporus males can also show the short-term steroid hormone responses to encounters described above for tree lizard and side-blotched lizard males. Male *S. undulatus* show T elevations in response to a series of staged laboratory encounters with other males during the breeding season, but not in response to similar staged encounters with females or other males outside the breeding season (Smith and John-Alder, 1999). Corticosterone levels in males are increased by both male and female encounters, while females do not show hormonal responses to either male or female encounters.

Species in the genus *Sceloporus* were also recently used to test the hypothesis that species/populations with short breeding seasons show a diminished glucocorticoid stress response, so that only massive stressors would derail their infrequent opportunity at propagation. This hypothesis was tested by exposing individuals to varied durations of handling stress followed by blood collection and hormone analysis. However, no differences in the corticosterone stress responses to the stressor were detected between a short-breeding-season, single-clutching species, *S. virgatus*, and *S. undulatus* and *S. occidentalis*, species with longer breeding seasons and multiple annual clutches, therefore providing evidence against this hypothesis (Hews and Abell Banik, 2013).

### 2.10.2.3.5 Other

Baird and Hews (2007) examined the relationship between T, DHT, corticosterone, and aggressive behavior in territorial (older) and nonterritorial (younger but mature) male common collared lizards (*Crotaphytus collaris*). These authors found that concentrations of the aforementioned steroid hormones do not vary between the morphs, or with behavioral expression. Interestingly, when neighbors died, aggressive display rates increased in the nonterritorial males, but rather than experiencing coinciding increases in hormone levels, these individuals actually experienced decreases in their circulating corticosterone and T concentrations (the latter was solely a trend, but in the opposite direction of the behavior). This finding emphasizes the complexity of steroidal regulation of behavior and a lack of a direct correspondence between aggressive display and circulating steroid levels.

This complex relationship between circulating steroid hormone levels and aggression is echoed in a study by Husak and Lovern (2014) examining the relationship between T and aggression across 18 *Anolis* species inhabiting four Caribbean islands. As in the genus *Sceloporus*, many cross-species comparisons are possible in the diverse genus *Anolis*. These researchers predicted that species with higher aggression levels would be those that possessed higher baseline T concentrations. Instead, on three of the four islands, the circulating concentrations of T were inversely correlated with aggression.

### 2.10.2.4 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. There are scattered observations of parthenogenesis in snakes, but no further studies have been reported.

Among the whiptail lizards (*Cnemidophorus* spp., the genus was recently renamed to *Aspidoscelis*) fully one-third of the species reproduce by obligate parthenogenesis. The best studied to date is the triploid desert-grassland whiptail, *Cnemidophorus uniparens*. This particular species arose from the hybrid mating between two sexual species and restriction analysis of mitochondrial DNA indicates that two-thirds of its genome comes from the little striped whiptail, *C. inornatus* (Densmore et al., 1989; Conant and Collins, 1998; Figure 15).

During the breeding season males of the little striped whiptail lizards will mount receptive females; neither males nor females exhibit the heterotypical sexual displays. However, the descendant parthenogen *C. uniparens* displays both male-like mounting behavior and female-like receptivity, termed ‘pseudosexual’ behavior during discrete phases of the ovarian cycle (Crews and Fitzgerald, 1980; Moore et al., 1985a,b; Figure 16). This behavior is identical, excepting intromission, with that observed during mating in its sexual ancestor, *C. inornatus*.

The hormonal regulation of both sexual (ancestral species) and pseudosexual (descendant parthenogenetic species) behaviors as well as the downstream gene expression in discrete brain nuclei has been extensively studied (Crews, 2005). In both the parthenogens and female *C. inornatus*, circulating levels of E2 increase during the preovulatory (PreOv or follicular) phase and reach peak levels at the time of ovulation; P levels, which are low during most of the postovulatory (PostOv) phase, are at their highest following ovulation and then decline rapidly; androgens are not detectable in the circulation at any time of the ovarian cycle (Moore and Crews, 1986). The only apparent exception is that in the unisexual species the levels of circulating E2 are lower (Moore and Crews, 1986; Moore et al., 1985b; Young and Crews, 1995).

Female *C. inornatus* will stand and be receptive to the mounting and copulation of sexually active males only when those females have large, preovulatory follicles, but will be aggressive toward males following ovulation (Lindzey and Crews, 1988b); receptivity can also be induced by exogenous estrogen in ovariectomized females but P does not induce mounting behavior (Young and Crews, 1995). In the parthenogens, PostOv individuals will approach and investigate another individual and attempt to mount. If PreOv, the individual will stand and be receptive to the mounting and pseudocopulation ensues. During pseudocopulation the male-like individual will swing its tail under that of the other individual, and assume a genus characteristic donut-like position that places their cloacal openings in apposition.

In the parthenogens the P surge following ovulation mediates the male-like pseudosexual behavior. Although androgens are not detectable in the general circulation (Moore et al., 1985b), the whiptail brain is capable of de novo neurosteroidogenesis (Dias et al., 2009) and may explain why T is more effective than DHT in stimulating both mounting behavior and PR abundance in the PVPOA of unisexual whiptails (Godwin et al., 2000; Wade et al., 1993). Implantation of P directly into the POA, but not in the VMH, will elicit mounting in ovariectomized parthenogens (Crews, 1988).
C. inornatus and C. uniparens that paternal ancestor is still under dispute, with some favoring ancestor of involved. The best-studied parthenogen is species. Indeed, in many instances, we know which species were arose fully formed from the hybrid mating of two sexual whiptail that reproduce by true parthenogenesis. The parthenogenetic species species of whiptail lizards are unisexual, consisting only of individuals female individuals that reproduce sexually. However, one-third of the 45 recently renamed Figure 15 Most whiptail lizard species (genus Cnemidophorus, recently renamed Aspidoscelis) 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.

Castrated male C. inornatus do not mount and copulate with females but will do so after androgen treatment (Lindzey and Crews, 1986). However, about 25–50% of castrated males will respond to exogenous P with both mounting and copulation (P-sensitive males), the remainder do not respond to exogenous P (P-insensitive males) (Lindzey and Crews, 1986, 1988a). This effect of P is mimicked by R5020, a nonmetabolizable progesterone receptor (PR) agonist, and abolished by the anti-progestin RU486, suggesting that progestins mediate this behavior at the level of PR rather than via progestin metabolites such as androgens (Lindzey and Crews, 1988a). Within a reproductive season, this phenotype is not plastic in that P-insensitive males do not become P-sensitive or vice versa. Progesterone-sensitive and progesterone-insensitive C. inornatus males differ in their neural substrates controlling sexual behavior (see Section 2.10.4.3).

2.10.2.5 Sex Steroids and Behavior: Other Reptiles

Reptiles display diverse relationships between circulating steroid hormones and reproductive behavior; see reviews 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.

2.10.2.5.1 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 T also rise during this period, although E2 does not significantly. Patterns in the loggerhead sea turtle (Caretta caretta) show similarities where P, T, and corticosterone all decline over the course of the mating season through repeated nesting episodes (Wibbels et al., 1990; Whittier et al., 1997). Sea turtles are most easily sampled during the nesting period when they emerge onto beaches. Less information is available on steroid levels during other seasons. Rostal et al. (1998) approached this problem by sampling from captive Kemp’s Ridley sea turtles (Lepidochelys kempi) under semi-natural conditions. Male Kemp’s Ridley turtles show T peaks several months prior to mating and these levels decline slightly by the mating season in March; they decline sharply following the cessation of breeding. Females exhibit peak levels of T, E2, and P at the time of mating. Both T and E2 decline sharply after mating, while P declines more slowly.

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

### 2.10.2.5.2 Crocodilians, Tuatara, and Amphisbaenians

The 21 species of crocodilians represent the most primitive extant members of the archosauromorph lineage that includes modern birds and the extinct dinosaurs. As with turtles, considerable information is available regarding steroid hormones and the process of TSD for crocodilians (represented by the American alligator, Alligator mississippiensis). 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 Sphenodontidae, are similarly limited. Female tuatara exhibit a prolonged reproductive cycle, carrying eggs in the oviduct for 6–8 months and nesting only once every 4 years on average (Cree et al., 1992). Tuatara appear to exhibit an associated reproductive pattern. Gametogenesis and T levels in males follow an annual cycle, being low during the winter, rising in the spring, and peaking in mid-summer to early autumn during the mating period. Female tuatara show elevated levels of E2 and T during vitellogenesis, which decrease after ovulation when P...

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**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. Redrawn from Crews, D., Coomber, P., Gonzalez-Lima, F., 1997. Effects of age and sociosexual experience on the morphology and metabolic capacity of brain nuclei in the leopard gecko (Eublepharis macularius), a lizard with temperature-dependent sex determination. Brain Res. 758, 169–179.
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 oviducal contractions (Guillette et al., 1991).

As when this topic was last comprehensively reviewed (Whittier and Tokarz, 1992; Moore and Lindzey, 1992), no information is available regarding the relationship of steroid hormones to either reproduction or behavior in amphisbaenians. However, it has recently been suggested that an interplay between steroids and social communication in amphisbaenians may exist, given that males respond with chemosensory and aggressive responses to squalene, a biochemical precursor to cholesterol and thus all steroid hormones, as they do to precloacal secretions (López and Martín, 2009).

### 2.10.3 Neuroanatomical Substrates of Sexual and Aggressive Behaviors in Reptiles

The neural pathways subserving sexual and aggressive 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. For instance, the final common pathway for male-typical mounting and intromission behavior appears to be the 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, and VMH in regulating female-typical, suites of reproductive behaviors. Examples of five such types of experiment are described below.

#### 2.10.3.1 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, T, 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 T in the brain of red-sided garter snakes. These studies, similar to those in mammals and other vertebrates (see Morrell and Pfaff, 1978), reveal substantial E2 binding in the POA, AH, amygdaloid nuclei, 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 T and DHT are very similar to patterns for tritiated E2, but there are some differences in pallial and the mesencephalic tegmental area.

Later, a series of studies explored the location and hormonal and social regulation of the mRNAs encoding the estrogen receptor-2 (ERz), AR, and PR in whiptail lizards (reviewed in Crews, 2005; Young and Crews, 1995). These studies elucidated the brain regions where each of these receptor mRNAs is expressed, variation in receptor mRNA abundances over the course of the ovarian cycle, species differences in expression levels and behavioral correlates of this expression, sex and species differences in the hormonal regulation of this expression, developmental influences on the sexual differentiation of hormonal responsiveness in receptor mRNA expression, as well as documenting social influences on ER- and PR-mRNA abundance in hypothalamic nuclei. Regulation of receptor expression is discussed in a later section, and the discussion that follows will be limited to the neuroanatomical distribution of hormone receptors.

As in other groups of vertebrates, reptiles show what Pfaff and coauthors termed a ‘lawfulness’ of steroid receptor distribution in the brain (Pfaff et al., 1994). Indeed, this conservation in distribution provides evidence of neural homologies as with the expression of AR in the lizard DVR and the avian magnocellular nucleus of the anterior nidopallium (MAN) (see below). In situ hybridization 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 (see below).

Strong ER mRNA expression is found in the whiptail 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 nuclei. 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 T, respectively, and it is possible that some of the T label was aromatized to E2 (see Wade, 1997, 2005) and then bound the ER. This is discussed at greater length below.

Progesterone receptor 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. Strong labeling is also found in the medial septum, lateral POA, central amygdala, AH, the posterior, dorsal and VMH, the lentiformis thalamus pars plicata, and the torus semicircularis. Lower levels of PR mRNA are evident in the lateral hypothalamic area, near the premamillary 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, medial POA, AH, IS, dorsolateral anterior nucleus, VMH, periventricular nuclei of the hypothalamus, and especially the premamillary nucleus (Young et al., 1994; Rhen and Crews, 2000; Figure 17). This distribution reveals differences from previously documented distributions in the brains of green anole lizards and garter snakes based on steroid autoradiography. Specifically, AR mRNA is more abundant than ER mRNA in the IS and ER mRNA does not occur in the external amygdala of whiptail lizards. Of particular interest from a comparative perspective is sexual dimorphism in expression...
of AR mRNA in the DVR of the lizard, which may have a function similar to that of MAN in songbirds (Young et al., 1994). 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). These workers found AR mRNA 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. Beck and Wade (2009a,b) also examined the expression of ERz mRNA in the embryonic, perinatal, and adult green anole forebrain, finding more mRNA in the adult female POA and amygdala than in adult males, and mRNA expression several times greater in the developing VMH than in the POA and amygdala of both sexes during development and in adulthood. Cohen et al. (2012) further examined the expression of ERß mRNA in green anoles, finding widespread forebrain expression, including the POA, VMH, and amygdala, with greater densities of ERß-expressing cells in the VMH and amygdala of females than males. Immunocytochemical studies such as those of Moga et al. (2000) have used an antibody directed at a conserved sequence

**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 three) in the right half of brain sections. Solid circles indicate heavily labeled cells and hollow circles indicate lightly labeled cells. Abbreviations: AC, anterior commissure; AME, nucleus externus amygdale; DH, nucleus dorsalis hypothalami; LFB, lateral forebrain bundle; LHA, lateral hypothalamic area; LPA, lateral preoptic area; MPA, medial preoptic area; NS, nucleus sphericus; NSL, nucleus septalis lateralis; 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, L.J., Lopreato, G.F., Horan, K., 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). J. Comp. Neurol. 347, 288–300.
in the N-terminal domain of the AR protein [residues 1–21 of the rat AR] to map AR-ir in 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. Androgen receptor immunoreactivity 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. Androgen receptor 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 nuclear staining in the medial POA, periventricular hypothalamus, and ventromedial, premammillary, and arcuate nuclei. AR-ir fiber staining occurred throughout the AH and POA and in a variety of other diencephalic areas. Females showed nuclear AR-ir in fewer areas than males, with nuclear staining only in the 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 here for S. undulatus could represent a species difference or a difference in the sensitivity of the immunocytochemical method employed relative to the in situ hybridization approach used in other studies. Male whiptail lizards do show higher levels of AR mRNA in the medial POA 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 medial POA, 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 colocalization of different receptor types within cells would be particularly useful.

### 2.10.3.2 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). Intracranial implantation of P rather than androgen is also effective in restoring courtship and copulatory behavior in a subset of C. inornatus males that are sensitive to intraperitoneal P implants (Crews et al., 1996b).

As with male-typical sexual behavior, 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.

### 2.10.3.3 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 examples of such differences in brain nuclei or soma sizes are present in reptiles.

#### 2.10.3.3.1 Sexual Dimorphisms

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 18). That is, castration of breeding animals results in both a reduction in the area of the POA, and an enlargement 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 sex.
with differences in soma size within these areas. Male \textit{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 \textit{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 \textit{C. uniparens} (Wibbels and Crews, 1994; Wennstrom and Crews, 1995; Wennstrom et al., 1999). This dissociation between brain and behavior indicates that, while sometimes useful, measurements of brain nucleus volume and soma size often do not reflect specific functions. This topic will be further addressed below when we discuss metabolic capacity, neurotransmitter function, and regulation of steroid hormone receptor expression.

Information is also 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 ‘push ups’ as in many male lizards and are also able to greatly extend a red-pigmented portion of skin on the ventral side of the neck, termed the dewlap, through flexion of the hyoid apparatus (Crews, 1975a); although female green anoles have similar pigmentation in the gular region and use this as an aggressive signal, neither the skin or the hyoid is as well-developed as in males (Crews, 1975b). Both of these signals are used in social contexts with dewlap extension as a courtship signal being shown only in males while female headbobs, with or without dewlap extension, are regarded as a signal of submission during male–female interactions (Crews, 1975b). The muscle primarily responsible for dewlap extension, the ceratohyoides 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 (AmbX/VIImv). Neurons in both brain regions are larger in males than in females (Wade, 1998). Motor neuron number does not vary by sex or across the breeding and nonbreeding seasons, but nerve cross-sectional area and both muscle fiber size and number are greater in males than females (O’Byrant and Wade, 1999). However, there is no consistent relationship between either the breeding/nonbreeding season or androgen treatment (T propionate) and these characteristics in males. Testosterone 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 T 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 AmbX/VIImv (O’Byrant 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’Byrant and Wade 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, 2007b). The same study also suggests that androgen-sensitive tissues in studs might be more responsive to T than those in duds, potentially explaining some of the behavioral variation.

In the tree lizard, males on average possess a larger POA and amygdala than females (Kabelik et al., 2006). A lateral portion of the ventromedial nucleus of the hypothalamus, which has been linked to aggression (Kabelik et al., 2008a), was also larger in males, although the overall nucleus did not differ between males and females. Testosterone level was shown to underlie some of this neural plasticity, with male tree lizards treated with T-filled Silastic implants possessing larger amygdaloid nuclei than controls or males receiving P-filled implants (Kabelik et al., 2008c; Figure 19).

Figure 19 (a) A depiction of the location, in unilateral coronal brain sections, of the lateral septum (LS), preoptic area (POA), nucleus sphericus (NS), amygdala (AMY), ventromedial hypothalamus (VMH), and habenula (HAB). (b) Compared to blank implant-treated subjects, testosterone but not progesterone treatment increased the average cross-sectional area of the NS and AMY in male tree lizards. Both testosterone and progesterone had been found to increase aggressive display in the same study. * denotes a significant difference between homogenous groups, with the latter denoted by lines above bars. Adapted from Kabelik, D., Weiss, S.L., Moore, M.C., 2008c. Steroid hormones alter neuroanatomy and aggression independently in the tree lizard. Physiol. Behav. 93 (3), 492–501.
The research described above focuses on species with the familiar pattern of GSD. The other end of the genotype—environment spectrum is represented by species that exhibit TSD. In the leopard gecko, both low and high incubation temperatures produce females while intermediate temperatures result in male determination and differentiation (Figure 4). 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 temperature. 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.

2.10.3.3.2 Seasonal Variation
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 2.11, Neuroendocrine Regulation of Reproductive Behavior in Birds by Ball and Balthazart; and Chapter 2.12, Neural and Hormonal Control of Bird-song by Schlinger and Brenowitz, this volume). Many reptiles also show seasonal reproduction.

Along with the sexual dimorphisms described above, Kabelik et al. (2006) also reported changes in tree lizard limbic nuclei volumes across the breeding season (Figure 20). 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 because the nucleus changes do not correlate well with behavioral changes, further emphasizing the lack of a direct correspondence between structure size and individual function.

Seasonal changes in limbic brain nucleus volumes have also been reported in the green anole, where male and female subjects from the breeding season possess larger POA and AMY volumes than those from the pre-breeding period (Figure 20). The figures are based on estimated marginal means following the inclusion of whole brain volume as a covariate. * denotes a significant difference between the three periods at p < 0.05, † denotes a strong trend at p = 0.056. Bars represent means ± one standard error. Adapted from Kabelik, D., Weiss, S.L., Moore, M.C., 2006. Steroid hormone mediation of limbic brain plasticity and aggression in free-living tree lizards, Urosaurus ornatus. Horm. Behav. 49, 587–597.
VMH volumes than do those from the nonbreeding season (Beck et al., 2008). Interestingly, while no volume differences were found across seasons in the amygdala, the number of neurons in this region was found to be greater in nonbreeding season relative to breeding season animals.

In the Canadian red-sided garter snake 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).

2.10.3.4 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 14C 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 21). Specifically, animals displaying male-like pseudocopulatory behavior showed a sixfold greater accumulation of 2-fluoro-2-DG in the medial POA than did animals showing female-like behavior. Conversely, individuals showing female-like receptive pseudosexual behavior exhibited a greater accumulation of 2-DG in the VMH.

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 garter snake males. 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; this finding is paralleled by results using immediate early gene products as markers of neural activity, as discussed below.

Radiolabeled 2-DG has also been used to study patterns of metabolic 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 22). 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) 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.

2.10.3.5 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 (Wheeler and Crews, 1978) and little striped whiptail lizards (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 (Kendrick et al., 1995). 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.

2.10.4 Neurochemical Bases of Sexual and Aggressive Behavior in Reptiles

The relationship of neurotransmitters and neuropeptides to sexual and aggressive behaviors in reptiles has received relatively little attention, but some progress has been made in understanding the neurochemical mechanisms underlying such behaviors in anoles, tree lizards, and whiptails. Furthermore, other signaling molecules such as nitric oxide (NO), as well as other regulatory molecules such as steroid converting enzymes, need to be considered.

2.10.4.1 Nonapeptides

Arginine vasotocin populations have been examined in a number of reptile species and generally exhibit strong homology to those found in other amniotes (Hattori and Wilczynski, 2009; Kabelik et al., 2013; Propper et al., 1992a). Some differences between species have been noted, but disagreement about reptilian neuroanatomy and differences in experimental procedures make it difficult to be certain of this variability, and further studies are needed to solidify conclusions. A possible link between sociosexual behaviors and AVT is suggested by Propper et al. (1992a) who report that AVT immunoactivity in green anoles is generally of greater intensity in males than females. Hattori and Wilczynski (2009) also found increases in diencephalic AVT immunoactivity in male green anoles that were the dominant individual in a stable dominance hierarchy, while decreased (from control groups) AVT immunoactivity was found in animals that held the subordinate role. Dunham and Wilczynski (2014) found that intraperitoneal injections of AVT in male green anoles decreased aggression in mirror trials (with one’s own reflection), but not aggressive interactions among size-matched males; however, administering AVT in this manner led to a concomitant increase in circulating glucocorticoids, making it unclear what was the causal variable to the reduction in aggression.

Immediate early genes, the most commonly examined being c-fos, are expressed in response to recent neural activation and can be visualized via in situ hybridization and immunohistochemistry. Like the 2-DG technique described above, visualization of the resultant mRNA or protein can be used to assess recent neural activity in specific brain regions. However, using immunofluorescence or other multilabel approaches, one can further colocalize this activity marker to specific populations of neurons. Kabelik et al. (2013) used immunofluorescence to colocalize AVT and c-Fos immunoactivity in male brown anoles (Anolis sagrei) and determined that AVT-producing cells were activated during both sexual and aggressive behaviors. However, all populations of AVT-producing neurons did not act in synchrony. In particular, AVT-immunoreactive neurons in the POA were activated primarily in sexual contexts, whereas populations in the BNST, paraventricular nucleus of the hypothalamus, and supraoptic nucleus were activated during both sexual and aggressive behaviors (Figure 23). The latter is somewhat at odds with findings in other amniotes, especially birds, where the BNST population tends to be activated in sexual contexts and inhibited in aggressive contexts (Goodson and Kabelik, 2009).
No sex differences were described in vasopressin-like or oxytocin-like (presumably AVT and mesotocin) immunoreactivity in the brain of the chameleon, although differences were found for females across the ovarian cycle (Bennis et al., 1995). This variation in AVT-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). In whiptail lizards, T administration also increases the number of AVT-IR neurons in the POA of female C. inornatus (Hillsman et al., 2007; Figure 24). There are a few reports in other reptiles regarding distribution of AVT immunoreactivity (e.g., the turtle (Pseudemys scripta) and snake (Python regius) (Smeets et al., 1990)).

![Image](74x411 to 299x721)

**Figure 23** Arginine vasotocin (AVT) neurons and fibers in the male brown anole brain: (a) preoptic area, POA; (b) suprachiasmatic nucleus, SON; (c) bed nucleus of the stria terminalis, BNST; and (d) paraventricular nucleus, PVN. (e) Neural activity, as assessed by colocalization with Fos 90-min post behavioral encounter, reveals that all of these populations of vasotocinergic neurons exhibit increased activation during social encounters in individuals that exhibited maximum behaviors for that category (biting of opponent during aggression trials, copulation during courtship trials). Note that activity within the parvocellular POA neurons was elicited specifically by sexual encounters, while activity within the magnocellular neurons of the PVN was elicited by aggressive encounters. Different letters above bars designate groups differing at p < 0.05. Other abbreviations: lfb, lateral forebrain bundle; III, third ventricle; oc, optic chiasm. Adapted from Kabelik, D., Alix, V.C., Burford, E.R., Singh, L.J., 2013. Aggression- and sex-induced neural activity across vasotocin populations in the brown anole. Horm. Behav. 63 (3), 437–446.

![Image](320x305 to 543x721)

**Figure 24** Arginine vasotocin immunoreactive cells (AVT-ir) and fibers in the periventricular preoptic area (PP) and the bed nucleus of the stria terminalis (BNST) in a Virago Cnemidophorus uniparens (aromatase inhibitor created males) receiving a blank implant (Top Panel). Other abbreviations: AC, anterior commissure; LFB, lateral forebrain bundle; OC, optic chiasm; SO, supraoptic nucleus. Bottom Left Panel: Testosterone treatment increases abundance of AVT-ir in the PP 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 PP 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, K.D., Sanderson, N.S.R., Crews, D., 2007. Testosterone stimulates mounting behavior and arginine vasotocin expression in the brain of both sexual and unisexual whiptail lizards. Sexual Devel. 1, 77–84.

Variation in AVT level with sex, reproductive state, hormone level, and seasonal cycle has also been detected in various neural populations of the tree lizard (Kabelik et al., 2008b). In general, males of this species also exhibited greater AVT...
immunoreactivity than did females, and AVT levels were higher when circulating T concentrations were increased (Figure 25). However, direct correlations were not found between AVT expression and aggression levels in these studies, suggesting that activation of AVT neurons (see c-Fos studies above) is more directly related to individual differences in social behavior expression than is AVT chemoarchitecture. Large-scale differences in chemoarchitecture may nevertheless underlie species differences in social behavior propensities.

Much less work has been carried out in reptiles on the nonapeptide mesotocin (nonmammalian homolog of oxytocin). Like AVT, mesotocin has been linked to egglaying, including the timing of nesting behavior in three-toed box turtles (Carr et al., 2008). However, recent work in brown anoles by Kabelik and Magruder (2014) also found positive correlations between sexual behavior and c-Fos colocalization in mesotocin neurons of the paraventricular nucleus of the hypothalamus. This finding suggests that mesotocin may play a similar role in social behavior regulation in reptiles as oxytocin does in mammals (Zingg and Young, Chapter 3.13, Oxytocin).

2.10.4.2 Monoamines

Like the changes in AVT expression associated with status in a dominance hierarchy (see above), dominance interactions have also been shown to influence monoamine metabolism in male lizards. For example, aggressive interactions increase plasma epinephrine (EPI) and norepinephrine (NE) 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 eye spot darkening are reduced by castration, suggesting an influence of T. 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) and the substrate for 5-HT, 5-hydroxytryptophan (5-HTP) (indicating enhancement of both 5-HT 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 this response.


In a recent study, Kabelik et al. (2014) examined c-Fos induction (a marker of recent neural activity) across catecholamine populations throughout the entire male brown anole brain (except for the retina and olfactory bulb). These researchers found evidence of widespread increases in Fos induction within several putatively dopaminergic populations following a sexual encounter with a female conspecific. These regions included portions of the POAH, a midline population (subdivision of the catecholaminergic A11 population) on the border of the diencephalon and mesencephalon, and the main tegmental midbrain populations (ventral tegmental area, substantia nigra, A8 population). Limited activation associated with sexual behavior was also detected in more caudal, nondopaminergic catecholamine populations. During male–male aggression encounters, Fos induction was also present in catecholaminergic neurons within the ventral tegmental area and the inferior raphe region in animals exhibiting intense aggression (biting of opponent rather than simply stereotyped display from a distance). The limited amount of Fos induction in dopaminergic neurons of brown anole males engaged in aggressive encounters, coupled with the findings of DA release during agonistic encounters or even a perceived social threat (Watt et al., 2007) in the closely related green anole, suggest complex regulation of DA release in social conditions; these brown anole males may have seen their opponents as not very threatening (they were size-matched, with opponents slightly smaller than focal males) and the involvement of DA release in limbic regions during aggression seems to depend on one’s dominance status and recognition of opponent, with some situations eliciting DA release, while others do not do so, or even inhibit DA release (Ling et al., 2010). Alternately, some catecholamine release may occur during aggressive encounters without activation of the cell soma and accompanying Fos induction (e.g., local release of neurotransmitters from vesicles already docked at release sites of axons).

Patterns of monoamine metabolism in mountain spiny lizards 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 males results in increases in both 5-HTP and 5-HIAA/5-HT ratios. These interactions also increase activity of the central DA and EPI systems. Plasma levels of NE and EPI also rise rapidly during restraint stress or following territorial interactions (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, 5-HT turnover, estimated from the ratio of 5-HIAA, a metabolite formed after 5-HT release, to 5HT 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). Testosterone injections (1.6–2.0 mg kg⁻¹) enhanced 5-HT turnover in the hippocampus, but not the medial amygdala. There were no changes found in several other brain regions or in the activity of other monoaminergic systems. The authors note the possibility that the injected T
Hormones, Brain, and Behavior in Reptiles

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Figure 26  Neuron size and abundance of tyrosine hydroxylase in the periventricular preoptic area of female whiptail lizards. Panel A: Tyrosine hydroxylase immunoreactive (Th-ir) cells in Ceramophorus uniparens (uni, black bars) are larger than those of female C. inornatus (ini, open bars), presumably reflecting ploidy. Panel 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 prevulatory stage (PREOV); females of the ancestral sexual species show no change in cell number. Mean ± SEM shown. After Woolley, S.C., Lydon, J., O’Malley, B.W., Crews, D., 2006. Genotype differences in behavior and the number of catecholamine producing cells between wild-type and progesterone receptor knockout mice. Behav. Brain Res. 167, 197–204.
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.

Serotonergic neurotransmission is involved in gating male- as well as female-like pseudosexual behavior expressed by the parthenogenetic whiptail *C. uniparens*. Increasing 5-HT levels in T-implanted *C. uniparens* via systemic administration of a combination of the monoamine oxidase inhibitor, clorgyline, and the precursor of 5-HT, 5-HTP suppresses male-like pseudocopulation (Dias and Crews, 2006; Figure 28). In contrast, administration of pCPA (a tryptophan hydroxylase inhibitor) decreases 5-HT 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, E2-injected *C. uniparens* was suppressed by injection of 5HT into the VMH (Dias and Crews, 2008). Pharmacological targeting of specific 5-HT receptors indicates that the 5-HT1A and 5-HT2A receptors are involved in male-like pseudocopulation and female-like receptivity, respectively. In situ hybridization used to measure 5-HT1A and 5-HT2A receptor expression

Figure 27  Tract tracing and tyrosine hydroxylase immunocytochemistry (TH-ir) in the medial preoptic area (mPOA) of male whiptail lizards. Left: Tracer combined with testosterone was implanted into the mPOA. TH-ir neuron in the MPOA labeled green, identifying the cells that may provide dopamine involved in modulation of male-typical copulatory behavior. Right: Retrogradely labeled cell in external amygdala which projects to the mPOA.

Figure 28  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 prevent the pCPA induction of mounting behavior. Data from Dias, B.G., Crews, D., 2006. Serotonergic modulation of male-like pseudocopulatory behavior in the parthenogenetic whiptail lizard, *Cnemidophorus uniparens*. Horm. Behav. 50, 401–409.
female-like receptivity is simultaneously suppressed). Pseudocopulation in androgen-implanted animals while with either hormonal state (e.g., the facilitation of male-like behavior associated with either hormonal state (e.g., facilitation of male-like pseudocopulation in androgen-implanted animals while female-like receptivity is simultaneously suppressed).

2.10.4.3 Nitric Oxide

Nitric oxide is produced in neurons from arginine by the enzyme nitric oxide synthase (NOS) and plays a critical role in both peripheral and central control of reproductive behavior. As indicated above, the POA is important for mediating mounting behavior both in male C. inornatus and pseudocopulatory behavior in C. uniparens; lesions in this region abolish mounting behavior in sexually active animals. Implants of DHT or P, but not E2, restore mounting behavior in gonadectomized animals (demonstrating the involvement of AR and PR, but not estrogen receptor or ER) (Godwin and Crews, 1997, 2002). As in other vertebrates, this brain region has high densities of steroid hormone receptors (see Section 2.10.3.1), receives inputs principally from chemical sensory systems, and projects to structures involved in copulatory behavior. The rostral PvPOA, the homolog to the mammalian anteroventral periventricular nucleus, or AVPv, contains abundant sex steroid-concentrating neurons and the greatest concentration of neuronal NOS mRNA (Crews et al., 2009; Sanderson et al., 2008). Inhibition of nNOS, the neuronal enzyme that produces nitric oxide, prevents androgen-induced mounting behavior in both mammals and whiptail lizards (Sanderson et al., 2005). Administration of T to both C. uniparens and female C. inornatus induces mounting behavior and enhances NOS expression, as indicated by NADPH diaphorase histochemistry (a marker for nNOS) and citrulline immunoreactivity (a marker of recent nitric oxide production) (Sanderson et al., 2008; O’Connell et al., 2011a,b, 2012).

Progesterone activates mounting behavior in both P-sensitive male C. inornatus and in the unisexual C. uniparens through convergence onto the NOS signaling system in the PvPOA (Figure 29). In this instance progesterone activates genes that in males of the sexual species are normally dependent on androgens. In mammalian systems AR and PR bind to and activate a common canonical steroid response element (Cato et al., 1987; Nelson et al., 1999). In addition to AR and PR, Class I nuclear receptors include the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Of these, only AR and PR, but not the GR and MR are able to induce luciferase activity via the selective sex-limited protein androgen response element (Denayer et al., 2010). In P-sensitive morphs this specificity of action is partly dependent on the cellular expression of the receptors. Thus, small changes in the regulation of steroid hormone receptors are capable of conferring on one hormone (e.g., PR) new functions previously restricted to the other (e.g., AR). The interaction of AR and PR determines the regulation of nNOS within the PvPOA of animals that display mounting behavior among both P-sensitive and P-insensitive morphs of the ancestral bisexual species, and in the descendant parthenogenetic species.

This responsiveness to the two different steroids and involvement in copulatory behavior suggest a possible involvement in the neural control of pseudocopulation in C. uniparens which normally display male-like pseudosexual behavior under the influence of P, 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 L-NAME, which inhibits NOS. Using a repeated-measures design, ovariectomized, hormone-treated lizards that were

![Figure 29](image-url) Model showing the possible interactions between steroid hormone receptors and neurotransmitter modulation of the preoptic area (POA) and ventromedial hypothalamus (VMH) in the unisexual whiptail lizard (Cnemidophorus uniparens). In both brain areas steroid hormones regulate the release of neurotransmitters, which then change the level of excitation/inhibition in the respective areas in a reciprocal fashion to result in either male-typical or female-typical copulatory behavior.
displaying robust pseudocopulatory behavior were treated with an intraperitoneal injection of 600 μg L-NAME in physiological saline 1 h before testing with a receptive stimulus animal, or 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 (see above) for the locus at which P 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 brains of parthenogenetic whiptails might enable P 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., 2005) and in males (Sanderson et al., 2006), and also at the time of the ovarian cycle when male-like copulatory behavior is displayed. Furthermore, analysis of citrulline production suggests that nitric oxide is synthesized more in the POA 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 (Figure 30) 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 T are tested at four time points following the display of sexual behavior, nNOS transcript abundance and parallel NADPH diaphorase histochemistry indicate that nNOS is upregulated by T in a pattern consistent with a role in mediating hormonal gating of copulatory behavior (Figure 31).

2.10.4.4 Steroidogenic Enzymes, Cofactors, and Receptors

Unlike in many mammals and birds, androgens seem to be the main regulators of male sexual behavior in lizards such as green anoles (Crews et al., 1978; Winkler and Wade, 1998). However, it has been documented that DHT is insufficient to fully restore courtship behavior in castrated males (Crews et al., 1978; Rosen and Wade, 2000) and that aromatase activity is greater in the brains of males than females during the breeding season, with males possessing more aromatase-expressing cells than females in the POA; these findings suggest that some involvement of estrogens is still likely in the regulation of male sexual behavior (Cohen and Wade, 2010, 2011). Latham and Wade
conducted an experiment where male green anoles were castrated and given combinations of T and/or E2, and concluded that while androgens may increase courtship displays and copulations, estrogens seem to play a role in increasing mount attempts (Latham and Wade, 2010). Interestingly, in this and other green anole studies, steroidial manipulations exert much greater effects in the breeding than nonbreeding season (e.g., Neal and Wade, 2007a,b). Hence, a series of studies has recently been conducted in the Wade lab to determine what variables facilitate the salience of these hormonal signals in the breeding season. These investigators have looked for changes in aromatase, 5α-reductase, AR, ER, steroid receptor coactivator-1, and cAMP response element-binding protein (CREB) that may underlie the seasonal change in steroid hormone salience, rather than being themselves regulated to different levels by seasonal fluctuations in hormone levels (Beck and Wade, 2009c; Cohen and Wade, 2012; Kerver and Wade, 2013, 2014, 2015). However, none of the variables examined to date seem to consistently vary in the POA across seasons, at standardized hormone levels, thus suggesting that other, still unknown, variables may underlie the seasonal change in male responsiveness to sex steroid hormones.

### 2.10.5 Regulation of Sex Steroid Hormone Receptors

A next step typically would be to study the receptor proteins. However, cross-reactivity of the antibodies available at the time made this impossible and the focus changed to cloning and sequencing reptilian steroid hormone receptor genes. In so doing, advancement in understanding hormone receptor gene expression in reptiles co-occurred with advances in rodents. While it is important to note that mRNA abundances do not necessarily reflect concentration of the corresponding protein, the ability to localize and compare relative abundance levels of the three main gonadal steroid hormone receptors in whiptail lizards has allowed a variety of questions related to sex and species differences in their regulation to be addressed. Recent methods in molecular techniques obviate this problem altogether.

**Figure 31** The effect of androgen on behavior, neurochemistry and gene expression in the male whiptail lizard (*Cnemidophorus inornatus*). Panel 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. Panel 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. Panel C: Relative nNOS transcript abundance in laser-microdissected fragments of PPA. Raw measures of NOS transcript abundance are expressed relative to 18S ribosomal RNA abundance and normalized to the mean value of the blank-implanted individuals. Panel D: Expression of nitric oxide synthase as inferred from NADPH diaphorase histochemistry. Presented are numbers of NADPH− 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, N.S.R., Le, B.D., Zhou, Z., Crews, D., 2008. Preoptic neuronal nitric oxide synthase induction by testosterone is consistent with a role in gating male copulatory behaviour. Eur. J. Neurosci. 27, 183–190.
Estradiol increases ER mRNA abundance in discrete brain regions in the whiptail lizards. Young and coworkers documented this using a 0.5 µg injection of estradiol benzoate (EB) and measuring ER mRNA abundance 24 h after administration. The EB effectively stimulates female-typical receptive behavior in parthenogenetic whiptail lizards (Young et al., 1995a) as well as increases ER mRNA in some regions (torus semicircularis, 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 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 (Young and Crews, 1995). 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 are 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. 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 Crews, 1980). Estradiol benzoate 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 32). Estradiol benzoate also effectively stimulates increases in PR mRNA in the POA of female C. inornatus, again with similar dosages being more effective in the parthenogenetic C. uniparens than in females of the sexual ancestor C. inornatus (Figure 33; 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 32  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.

Figure 33  Evolution of a novel neuroendocrine controlling mechanism. Progesterone receptor mRNA levels in the PoPOA 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. From Godwin, J., Crews, D., 1999. Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: regional and species differences. J. Neurobiol. 39, 287–293.

While E2 increases PR mRNA in both the VMH and POA of female and parthenogenetic whiptail lizards, exogenous P inhibits both female-typical receptive behavior and decreases estrogen-stimulated ER and PR mRNA in the VMH (Godwin et al., 1996).
et al., 1996). This effect of P on both receptivity and ER and PR mRNA abundance is similar to patterns in well-studied rodent models. In contrast, exogenous P has no effect on PR mRNA abundance in the periventricular POA in this experiment. Neither the effective induction of female-typical receptive behavior or 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. In contrast, male C. inornatus castrated for longer periods (6 weeks) showed PR mRNA responses to estrogen that were not different from females (Wennstrom and Crews, 1998). Females implanted with T, however, did not show an attenuation of the female pattern of responsiveness. These results indicate that maintenance of the male-typical pattern of nonresponsiveness requires the activational effects of T 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 P delivered in Silastic capsules implanted intraperitoneally in reinstating sexual behavior following castration (Lindzey and Crews, 1992). Males classified as P-sensitive are also significantly more likely to respond to intracranial implants of P (directed at the POA) than 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 P. Progesterone-sensitive males display lower abundances of PR mRNA in both the medial and periventricular portions of the POA, but higher abundances of AR mRNA in the medial POA, external amygdala, and 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 T or DHT reveals a diversity of effects, suggesting that gonadal sex, aromatization, and gene dosage (ploidy) all influence steroid receptor mRNA response (Godwin et al., 2000) (see also Young et al., 1995a for PR mRNA). For example, males have higher AR mRNA in the medial POA than females of either species and these levels decrease with T treatment in males, but not in females. In contrast, ER and PR mRNA levels in the VMH are higher with androgen treatment, but these effects do not differ by sex. There also are species effects in that the triploid parthenogen shows higher steroid receptor mRNA abundances overall than the diploid sexual females. Finally, aromatization of T to estrogen is likely important in some regions. Progesterone receptor mRNA in the periventricular POA is increased in both males and females by T, 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 in 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 colocalization of receptor types within neurons and crosstalk between signaling systems, exploring the influences of other mediators (e.g., corticosteroids, thyroid hormones, neurotransmitters) on receptor regulation, and characterizing the downstream effects of steroid receptor activation.

2.10.6 Conclusions and Future Directions

Reptiles enable study of the neuroendocrine mechanisms underlying of 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. In this manner, study of species with dissociated reproductive tactics (including nonreptilian species such as birds and mammals) and unisexual species suggests 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 reexamine certain assumptions in behavioral neuroendocrinology. One such example concerns the idea that P is a ‘female-specific’ hormone with no function in males. Experiments with four lizard species have demonstrated that P is vital to the display of male copulatory behavior in lizards and, further, that androgen and P synergize in males, much like E2 and P synergize in females to facilitate sexual receptivity; subsequent studies with mice and rats have revealed similar roles for P and its receptor in male sexual behavior in mammals.
Factors during embryogenesis, and in still others it is the social sex chromosomes at fertilization, in others it is environmental determination are conserved. What differs is the trigger; in some it is in each individual. That is, the species may differ in their 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 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 TSD, gonadal sex is determined by incubation temperature during the middle of development. In hermaphroditic species such as sex-changing fish, 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? 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 the above observations. This framework 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 feminizing individuals suggests this to be the case.

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 sexual 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 archosaururomorph 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 activity, neurotransmitter metabolism, and the regulation and actions of steroid hormone receptors all show the value of these approaches. Not only are such investigations important to further our understanding of species and sex differences but also of individual differences. The mechanisms that generate individual variation are an important focus across modern biology in general. 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.

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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