Arousal is a term that may be hard to characterize for vertebrates as distant to humans as reptiles. However, the comment from Judge Potter Stewart about hard-core pornography comes to mind. One certainly ‘knows it when one sees it’. In many reptilian species, arousal is clearly observed in two contexts directly associated with reproduction — sex and aggression. The former is often divided into appetitive (or courtship) and consumatory (copulatory) behaviors. The latter might take the form of territorial defense or competition between males for access to mates.

Relatively few reptilian species have been investigated extensively in this field. However, lizards in particular provide terrific opportunities for elucidating mechanisms from a comparative perspective. They exhibit diverse mating strategies and utilize multiple sensory modalities for communication, which is critical in sexual and aggressive contexts. A variety of species use chemical cues. They are primarily processed by the vomeronasal organ, and commonly...
involves secretions from femoral glands on the ventral thigh that are deposited on the substrate. These secretions can increase or change in composition in response to testosterone (T), and are used for species identification, as well as conveying information about breeding levels of androgen in males (Houck, 2009). For example, male whiptail lizards (Cnemidophorus genus) use this type of signal during their courtship sequence. When encountering a female, a male moves his pelvis rapidly back and forth along the ground, releasing femoral pore secretions, a behavior that has been called a “swiggie walk” (Lindzey and Crews, 1986). Some lizards employ vocalizations, such as the tokay gecko (Gekko gecko). These animals produce croaks or calls, and as with chemical cues, androgens are likely important in both the production and processing of these signals that are used for communication associated with courtship and aggression (Tang et al., 2001). Other species described in this review utilize visual signals, including particular patterns of coloration and movement (see later). In all cases, the production of these signals by males provides a clear indication of motivation to display behaviors associated with reproduction. Much of the research in this area has focused on males, at least in part because parallel levels of arousal can be difficult to detect in females. Female lizards generally do not have distinct sexual behaviors, such as ear wiggling or the receptive lordosis posture displayed by rodents. Receptivity in lizards is largely obvious to human observers simply by their lack of escape behavior which allows a male to successfully mount and intromit.

One of the species that relies heavily on visual cues is the green anole (Anolis carolinensis), the lizard with the longest history of research in behavioral neuroendocrinology. Publications are available from as early as the 1880s (Monks, 1881). Detailed behavioral research was conducted in the 1930s and 1940s, and investigations into neural and endocrine mechanisms began in the 1970s. This lizard is a member of a large and diverse genus, with approximately 400 species mainly occurring in the Caribbean, and Central and South America (Nicholson et al., 2005; Schwartz and Henderson, 1991). The green anole’s native range is among the northern-most, including much of the southeastern United States. This review will focus on the neural and endocrine mechanisms mediating motivated behaviors in this species, with comparisons made to research in other lizards.

**Hormones and Behavior**

**Anoles**

A prominent feature of many anole species is a dewlap, or throat fan. This structure is highly diverse, with most species having a unique combination of color, pattern, and size (Nicholson et al., 2007). In green anoles, it is red. In many species it is larger in males than in females, but substantial variability exists (Johnson and Wade, 2010). The extension of the dewlap (Fig. 1A), which commonly occurs in conjunction with a series of head-bobs and/or push-ups, is a clear indication of arousal. This structure is used by males both in displays of courtship toward females and territorial aggression toward other males; in the latter context a black spot behind the eye is also visible, and males frequently raise a crest along the dorsal surface of their heads and necks (Fig. 1C).

The sequence of social behaviors displayed by male green anoles is stereotyped and easily observed under both field and laboratory conditions (Crews and Greenberg, 1981; Decourcy and Jenssen, 1994; Greenberg and Noble, 1944; Greenberg, 2003). Following courtship, a male green anole will mount the female, maneuver his tail under hers, erect one of two independently controlled, bilateral hemipenes from inside his cloaca, intromit and ejaculate (Crews, 1980; Fig. 1B). During courtship and copulation (Fig. 1B), female green anoles may bob their heads in a manner similar to males, but generally without dewlap extensions (Nunez et al., 1997). Females do not have a characteristic posture, but only allow the male to mount if receptive. They may also bend their necks to facilitate the male’s biting onto the skin at the back of the females’ necks (Crews, 1980).

Green anoles breed seasonally, reproducing in the field from about April through July. Both long days and warm temperatures contribute to gonadal growth (Licht (1967a,b), but see Lovern et al. (2004b)). The gonads become refractory to environmental conditions in late summer, followed by a period of modest testicular (although minimal ovarian) growth during the winter. In the spring, gonadal secretions activate the display of sexual behaviors.

As is common among vertebrates, castration reduces the display of sexual behaviors, and treatment with T prevents or reverses that effect (Adkins and Schlesinger, 1979; Crews et al., 1978; Rosen and Wade, 2000; Winkler and Wade, 1998). However, unlike avian and mammalian species in which neural metabolism of T into estradiol (E2; via the aromatase enzyme) or 5α-dihydrotestosterone (DHT; 5α-reductase) is important for male courtship and/or copulation (Ball and Balthazart, 2002; Hull et al., 2002), in male green anoles T itself is the most potent activator of these behaviors. This characteristic is somewhat surprising, as it has been reported that DHT has a higher affinity for androgen receptors (ARs) than T, at least in human prostate (Wilbert et al., 1983). The ligand binding domain of the green anole androgen receptor (Ensemble Genome Browser) is 97% identical to the human amino acid sequence.

The importance of the role of adult T is supported by a variety of hormone replacement studies. Several have documented that T activates sexual behavior in castrated adult males, an effect that is greater in the breeding than in the non-breeding season (Neal and Wade (2007b) and references therein). Systemic treatment with E2 and inhibition of E2 synthesis have generally had little effect on male sexual behavior in green anoles (Crews et al., 1978; Mason and...
Adkins, 1976; Winkler and Wade, 1998). However, E2 can increase mount attempts specifically, suggesting that it may be associated with motivation to copulate (Latham and Wade, 2010). DHT alone is not sufficient to activate the full expression of male reproductive behaviors, although treatment with a 5α-reductase inhibitor suggests that metabolism of T into DHT is required for maximal behavioral performance (Rosen and Wade, 2000). Similarly, in brown anoles (A. sagrei), E2 does not activate sexual behaviors in castrated males to levels seen in intact males (Tokarz, 1986).

While the association has not directly been made, it is possible that T during embryonic development might also have a role in the organization of male sexual behaviors or other sexually dimorphic functions. This T could come from at least two sources. Embryos appear to produce T during the latter half of egg incubation (Lovern and Wade, 2003a). However, a sex difference in yolk T is present even earlier—it is increased in male compared to female eggs on the day they are laid and even while they are still in the oviduct (Lovern and Wade, 2003b). We recently demonstrated that maternal steroids can be transferred into eggs during this developmental period inside the oviduct (Cohen and Wade, 2010a).

In female green anoles, both E2 and T play important roles in facilitation of receptivity. For example, E2 alone can activate their sexual behaviors (Winkler and Wade, 1998), and sub-threshold doses with progesterone produce a similar effect (Wu et al., 1985). Administration of an anti-estrogen inhibits E2-facilitated receptivity (Tokarz and Crews, 1980). T activation of receptivity is at least partially due to aromatization (Winkler and Wade, 1998). Parallel to patterns observed in males, circulating T is greatest when females are most likely to display sexual behavior (Lovern and Wade, 2001).

Research on the hormonal regulation of aggressive behavior has been conducted in two species of anoles. In the green anole (A. carolinensis), both corticosterone and androgen are associated with agonistic encounters. For example, males of this species will respond to staged encounters with another animal or video images of conspecific aggression with an increase in corticosterone (Summers et al., 2005). Similarly, a male green anole’s own aggressive behavior may cause an elevation in plasma androgen (Yang and Wilczynski, 2002). T given to castrated animals will increase dewlap extensions directed at stimulus males, an effect that is eliminated by treatment with an aromatase inhibitor. This latter point is a bit difficult to interpret, as E2 alone had no effect in the same context, but perhaps E2 plays some facilitatory role even if it is not sufficient alone (Winkler and Wade, 1998). Data from a few studies (reviewed in Summers et al., 2005) suggest that males likely to be dominant have relatively high baseline corticosterone levels prior to agonistic interactions, and in early phases of an encounter glucocorticoids permit full, vigorous behavior (although more prolonged exposure might be inhibitory). In the brown anole (A. sagrei), relatively long term corticosterone treatment inhibits aggressive behavior as well as circulating levels of T (Tokarz, 1987). In parallel, the frequency of aggressive dewlap displays in response to visual exposure to a stimulus male is greater in the breeding than in the non-breeding season and is increased with T administration (Tokarz et al., 2002), suggesting that this hormone facilitates agonistic behavior. However, activational effects of T on these displays are not observed when males are exposed to their own image in a mirror, which elicits aggression (Cox et al., 2009).

Glucocorticoids have a variety of influences on neurotransmitter/neuropeptide systems (Summers et al., 2005). In green anoles, dominant males (established over the course of 10 days) have more cells in the preoptic area (POA) that are immunoreactive for arginine vasotocin than their subordinate counterparts, and subordinate males have fewer compared to males housed alone or with a female for the same duration (Hattori and Wilczynski, 2009). This difference was not related to plasma T evaluated at the end of this period, but earlier measures were not taken and corticosterone was not investigated.

Other lizards

Whiptail lizards (Cnemidophorus genus) provide a unique natural experiment for investigating mechanisms regulating reproductive behavior. The most extensively studied species in this regard include C. inornatus and C. uniparens (reviewed in Crews and Moore, 2005). The former is a gynochoristic (sexually reproducing) species consisting of both males and females. The latter is its direct descendant and is parthenogenetic (all-female, reproduces by cloning and is triploid—XXX). Similar to green anoles and other vertebrates, female receptivity is activated by E2, and male sexual behaviors (which include assuming a donut-shaped copulatory posture around the female) are activated by T. Surprisingly, the parthenogens display behaviors common to both females and males of the ancestral species—the former are generally displayed prior to ovulation when E2 levels are high, and the latter after ovulation when progesterone is elevated. Progesterone can also activate sexual behavior in some male C. inornatus, suggesting a potential opportunity for the mechanism to have evolved. However, progesterone modulation of male sexual behavior is not unique to this genus. While high, pharmacological doses can be inhibitory, progesterone can synergize with androgen to facilitate male sexual behavior in both green anoles and roidents (Witt et al., 1994).

In free-living mountain spiny lizards (Sceloporus jarrovi), T given to castrated males during the autumn breeding season fully restores aggressive and sexual behavior. However, identical manipulations during the summer non-breeding season are not as effective. Thus, as with green anoles, environmental factors appear to influence the ability of T to activate behavior. Seasonal differences in plasma T parallel levels of territorial aggression/defense, but rapid post-encounter increases in aggression are not caused by simultaneous changes in plasma T (Moore, 1988). Thus, while a sufficient level of T is likely important for masculine behaviors, other factors clearly modulate the responses.

The nature of these mechanisms impacting the display of motivated behaviors other than (or in addition to) adult hormones is diverse. For example, in leopard geckos (Eublepharis macularius), early environmental conditions are key. Members of this species have no sex chromosomes (Viets et al., 1993), and the development of ovaries vs. testes depends on the temperature of egg incubation, such that high and low temperatures produce gonadal females, whereas intermediate temperatures produce variable sex ratios. Individuals do not develop intersex gonads, but the intermediate incubation temperatures do affect behavior (reviewed in Crews and Moore, 2005). For example, females from eggs incubated at temperatures that produce a higher proportion of males (male-biased) are more aggressive than females from a female-biased temperature, and males from female-biased temperatures are less aggressive than those from male-biased temperatures. Incubation temperature also affects sexual responses. Females from a male-biased temperature are less attractive to males than those from female-biased temperatures. This early developmental experience also influences adult responses to T. This species uses chemical cues, but males also vibrate their tails to create a buzzing sound when they detect a female. While intact females appear to display this behavior, ovarioctomized females from male-biased temperatures that have been treated with T will display the behavior toward female stimulus animals.

Several groups of lizards exhibit alternative reproductive strategies which are associated with particular morphological characteristics. For example, within individual populations the dewlaps of tree lizards (Urosaurus ornatus) can be fully blue, orange or yellow, or combinations of blue/yellow or blue/orange. Males with orange/blue dewlaps are aggressive and territorial, whereas those with simply orange dewlaps are less aggressive and nomadic. These phenotypes are organized in development, such that post-hatching T facilitates the more aggressive morph and castration increases the probability of development into the non-aggressive morph. Adrenal progesterone may also play a role. Morph differences are not regulated by adult
levels of T or corticosterone. However, some plasticity does exist. The non-aggressive orange-throated males can switch between a sedentary satellite strategy and a more nomadic strategy in response to local environmental conditions. A rise in corticosterone under stressful circumstances inhibits T, resulting in reduced site fidelity (Thompson and Moore, 1992; reviewed in Crews and Moore, 2005). Arginine vasotocin was investigated in this species, as in green anoles (Kabelik et al., 2008a). Here, it appears that T regulates immunoreactivity in limbic brain regions including the bed nucleus of the stria terminalis and paraventricular nucleus. However, correlations between the immunoreactivity and aggression were not detected.

Multiple throat color phenotypes also exist in side-blotched lizards (Uta stansburiana), and these are genetically correlated within the sexes (Sinervo and Zamudio, 2001). Three plain color morphs—orange, yellow and blue—are present, along with intermediate phenotypes. Among males, the presence of orange results in a high level of aggression. Yellow-throated males are female mimics and non-territorial. Blue-throated males avoid cuckoldry by mate-guarding, but are less successful in defending their territories against the territorial. Blue-throated males avoid cuckoldry by mate-guarding, but are less successful in defending their territories against the

relationships, as high aggression in orange throats is associated with a more nomadic strategy in response to territory loss (Ball and Balthazart, 2002; Blaustein and Erskine, 1978). Activity of the estrogen synthetic enzyme aromatase is relatively high in dissections including the hypothalamus and POA. Within the POA specifically, the estimated total number of aromatase mRNA expressing cells is greater in males than in females, and the density of these cells is increased in the breeding compared to non-breeding season. In the VMH, the density of these cells is greater in females than in males (Cohen and Wade, submitted for publication). These results are consistent with E2's (limited) role in male copulation (Latham and Wade, 2010). The activity of this enzyme is increased in whole brain homogenates from males compared to females and is greater in breeding than in non-breeding males (Rosen and Wade, 2001). This activity is specifically regulated by T; in gonadectomized animals, the hormone induces an increase in males, but not in females, and it does so only in the breeding season. Whole brain activity of 5α-reductase, which catalyzes the conversion of T into DHT, is also increased by T in males. However, this effect occurs in both the breeding and non-breeding seasons (Cohen and Wade, 2010b). This result conflicts a bit with data from unmanipulated animals which indicated that the activity of this enzyme in whole brain homogenates did not differ between the sexes or seasons (Rosen and Wade, 2001). Further work on regional distribution and regulation of the enzymes might shed light on how steroid metabolism may influence particular functions.

Forebrain

Anoles

Steroid hormones act in particular regions of the forebrain to facilitate the behaviors. In green anoles, far more is known about the neural control of male than female sexual behaviors. Lesions of the anterior hypothalamus—preoptic area (AH-POA; Wheeler and Crews, 1978) and ventromedial nucleus of the amygdala (AMY; Greenberg et al., 1984; equivalent to the posterior portion of the medial AMY, Bruce and Neary, 1995) inhibit masculine courtship and copulation. Interestingly, results from direct implantation of steroid hormones into the AH-POA of castrated males produces different effects than systemic treatments (described earlier). While their effects are limited when administered systemically, DHT and E2 in implanted directly this area can facilitate male courtship (Crews and Morgentaler, 1979). Manipulations with this degree of specificity have not been conducted in female green anoles, but destruction of the basal hypothalamus impairs receptivity (Farragher and Crews, 1979). Thus, it is quite likely that, as in other vertebrates (Ball and Balthazart, 2002; Blaustein and Erskine, 2002), the ventromedial hypothalamus (VMH) is critical for this function.

Further support for the role of steroid hormones in limbic regions lies in the distribution of their receptors. We detected relatively high levels of mRNA and/or protein for AR in areas including the POA, AMY, and VMH of adult males and females during the breeding season (Rosen et al., 2002). Estrogen receptor alpha (ERα) mRNA is also expressed in these regions (Beck and Wade, 2009a,c), although the level in the VMH is substantially greater than in the POA and AMY. This regional difference is also detected around the time of hatching (Beck and Wade, 2009b). In general, the pattern is consistent with a role of E2 in the VMH in facilitation of female receptivity, as well as with the limited effect of this hormone in the regulation of male reproductive behaviors. In unmanipulated animals, expression of both AR and ERα mRNAs is sexually dimorphic. Across the forebrain regions examined, AR is greater in females than in males (Rosen et al., 2002). Similarly, ERα mRNA is increased in females in the POA and VMH (Beck and Wade, 2009c). We recently cloned green anole ERα, and are evaluating its expression in the brains of intact adult males and females during the breeding season (Cohen, Roach and Wade, unpublished).

Expression of steroid metabolizing enzymes in general follows patterns similar to other vertebrates (Ball and Balthazart, 2002; Lephart, 1996). Activity of the estrogen synthetic enzyme aromatase is relatively high in dissections including the hypothalamus and POA. Within the POA specifically, the estimated total number of aromatase mRNA expressing cells is greater in males than in females, and the density of these cells is increased in the breeding compared to non-breeding season. In the VMH, the density of these cells is greater in females than in males (Cohen and Wade, submitted for publication). These results are consistent with E2's (limited) role in male copulation (Latham and Wade, 2010). The activity of this enzyme is increased in whole brain homogenates from males compared to females and is greater in breeding than in non-breeding males (Rosen and Wade, 2001). This activity is specifically regulated by T; in gonadectomized animals, the hormone induces an increase in males, but not in females, and it does so only in the breeding season. Whole brain activity of 5α-reductase, which catalyzes the conversion of T into DHT, is also increased by T in males. However, this effect occurs in both the breeding and non-breeding seasons (Cohen and Wade, 2010b). This result conflicts a bit with data from unmanipulated animals which indicated that the activity of this enzyme in whole brain homogenates did not differ between the sexes or seasons (Rosen and Wade, 2001). Further work on regional distribution and regulation of the enzymes might shed light on how steroid metabolism may influence particular functions.

A number of parallels exist between the morphology of forebrain regions controlling reproductive behavior in green anoles and the degree to which behaviors are displayed. For example, the volume of the POA is greater in intact males than in females, and the volumes of the POA and VMH (but not AMY) are greater during the breeding than in the non-breeding season in both sexes (Beck and Wade, 2008a). Interestingly, the nature of some of these results on volume differences is influenced by the presence of testis and ovaries, and can also be modulated by E2 treatment in gonadectomized animals (Beck and Wade, 2009a), suggesting that gonadal hormones may regulate morphology somewhat independently of behavior.

A variety of other dimorphisms have been detected in the forebrain of green anoles. For example, somata in the POA and AMY of breeding anoles of both sexes are significantly larger than during the non-breeding season (O'Bryant and Wade, 2002a). Interestingly, in both regions cells are larger in gonadectomized males than females during the non-breeding season, regardless of whether they were treated with T (O'Bryant and Wade, 2002a). T-treatment of gonadectomized adult green anoles increases soma size in the POA and AMY, and in the AMY it does so to a greater extent in the breeding season.
than in the non-breeding season (Neal and Wade, 2007b). The size of these cells is also correlated with individual differences in the rate of dewlap extension in unmanipulated males (Neal and Wade, 2007a). While the estimated total number of neurons does not differ between the sexes or seasons in the POA or VMH of intact anoles (Beck and Wade, 2008a), in gonadectomized animals more cells are present in the female than in male VMH (Beck and Wade, 2009a). This result was influenced by a female-specific increase in cells in the non-breeding season. Like the aforementioned volume data, collectively these results on cell size and number indicate the potential for some effects of steroid hormones that are parallel for brain and behavior, as well as some gonadal influences on brain morphology that are quite different from the hormonal regulation of behavior. In parallel, a greater number of cells exists in the AMY during the non-breeding than the breeding season, in both unmanipulated and gonadectomized animals, regardless of whether they were administered E2 (Beck and Wade, 2008a, 2009a). These results suggest that some feature(s) of non-breeding environmental conditions facilitates the addition or survival of cells in this region.

Other lizards

In whiptails, the forebrain regions (AH-POA and VMH) involved in endocrine mediation of masculine and feminine sexual behaviors are the same as in other vertebrates (Crews and Moore, 2005), and they are sexually dimorphic in a manner that parallels behavior. In breeding C. inornatus, the AH-POA is larger in volume in males and the VMH is larger in females (Crews et al., 1990). The size of these areas is plastic in adult males, such that they become feminine outside of the breeding season or following castration. This effect is not detected in the parthenogens (Wade and Crews, 1991). It is mediated by T, such that T replacement in castrated males reinstates the masculine morphology and display of sexual behaviors. Interestingly, T also activates male-like sexual behaviors in female C. inornatus and C. uniparens, but does not change the volume of their AH-POAs or VMHs (Wade et al., 1993). Thus, while hormonally mediated parallels between brain and behavior exist in males, they are dissociated in females—masculine-sized brain regions are not required for the display of male-like behavior.

Mapping and extensive work on the regulation of steroid receptor mRNA expression has been conducted in whiptail lizards (reviewed in Godwin and Crews, 2002). In general, expression patterns of mRNAs from genes encoding AR, ERα, as well as progesterone receptor, are similar to other vertebrates and include the limbic forebrain. Some patterns of regulation are also consistent. For example, E2 stimulates progesterone receptor mRNA in the female VMH (in both the sexually reproducing and parthenogenetic species). However, a number of sex, species and regional differences exist that suggest a complex set of mechanisms that might influence the effectiveness of hormone–receptor interactions in the regulation of motivated behaviors in this genus.

In leopard geckos, the embryonic environment plays a larger role than gonadal sex in regulating the morphology of areas important to reproductive behavior (Coomber et al., 1997). The volume of the POA in males from a male-biased incubation temperature is larger than that of males from a female-biased temperature. In parallel, the VMH is smaller in males from a male-biased temperature than those from a female-biased temperature. Differences have not been detected between the sexes within incubation temperatures.

Two experiments on tree lizards investigated relationships between morphology of limbic brain regions and aggression. A field study (Kabelik et al., 2006), demonstrated a variety of sex and seasonal differences in the volume of areas including the POA, AMY and a portion of the VMH. However, while aggression levels were related to T, the sizes of these and other brain regions were not associated with frequency or intensity of aggression. The morphology also did not differ between the two male morphs investigated, orange and orange/blue. In addition, adult plasticity in the structures (at least at the level investigated) appears independent of T-induced changes in aggression. A lab study in which hormones were manipulated (Kabelik et al., 2008b) also documented a lack of correlations between forebrain morphology and aggression. Here, progesterone altered aggression independently of morphological changes in the limbic forebrain. Positive relationships were detected between both T and aggression and between T and morphology, but the sizes of brain regions were not correlated with the behavior. These studies provide further support for complex relationships between hormones and aggression (see earlier), and indicate that at least gross morphology of some forebrain regions is not associated with agonistic behavior.

Neuromuscular systems in anoles

More clear relationships between morphology and function exist in the structures directly involved with distinct movements. Lizards are a particularly useful group for investigating these associations because, unlike other vertebrates, they possess two neuromuscular circuits known to be involved in the regulation of the suite of reproductive behaviors—one controlling dewlap extension used in courtship and agonistic encounters, and another regulating copulatory organ function. Among reptiles, detailed work of this sort has really only been done in anoles. However, parallels exist with the neuromuscular systems regulating copulation in male rodents (Breedlove and Arnold, 1981; Forger and Breedlove, 1987) and the vocalizations of fish (Bass and Marchaterre, 1989), frogs (Kelley et al., 1988; Tobias et al., 1993), and songbirds (Wade and Buhlman, 2000; Wade et al., 2002).

Sex differences

The mechanics of dewlap extension have been studied in two species of anoles, A. carolinensis and A. equestris (the knight anole, native to Cuba). Contraction of the bilateral ceratohyoid muscles in the throat cause movement of three pieces of cartilage on each side of the animal (reviewed in Wade (2005), see Fig. 2). Among these, the 2nd ceratobranchi (which normally lie flat, running from under the chin to mid-chest) bow out, extending the throat fan (Bels, 1990; Font and Rome, 1990). Motoneurones innervate the ceratohyoid muscles ipsilaterally through the combined IX + X cranial nerve. These motoneurones are located in the caudal brainstem in the vagal component of the nucleus ambiguus (AmbiX) and the region containing the glossopharyngeal portion of the nucleus ambiguus and the ventral motor nucleus of the facial nerve (AmbiX/VIMv) (Font, 1991; Wade, 1998).

Consistent with androgens playing more important roles than estrogens in male reproductive behaviors, 5α-reductase activity is relatively high in the brainstem (Wade, 1997), which is the general region where dewlap motoneurons are. We have cloned both types I and II (Cohen and Wade, unpublished) and are evaluating neural mRNA expression in adult males and females in and out of the breeding season. However, at present nothing is known about specific patterns of expression in dewlap motoneurons.

Overall, the dewlaps of adult male green anoles are three to seven times the size of those in females (Jensen et al., 2000). Males tend to extend these structures more often than females do, even when administered equivalent amounts of T after castration (Adkins and Schlesinger, 1979; Mason and Adkins, 1976; Winkler and Wade, 1998). In parallel, the length of the 2nd ceratobranchial cartilage and cross-sectional areas of motoneuron somas, as well as the nerve containing their axons, are larger in males (O’Bryant and Wade, 1999; Wade, 1998). Dendritic arborization of dewlap motoneurons does not differ between the sexes during the breeding season (O’Bryant and Wade, 2000), however. Mass of the ceratohyoid muscles is greater in males than in females, which is at least partially due to increased size of individual fibers (O’Bryant and Wade, 1999); neuromuscular
junctions are also larger in males than in females (O’Bryant and Wade, 2002b). In a laboratory study of males, the size of these muscle fibers is correlated with the frequency of dewlap extension in a sexual context (Neal and Wade, 2007a). However, in a recently completed analysis of the relationship between behavior and dewlap-related structures in free-living green anoles, this pattern was not as obvious (Johnson et al., submitted for publication). In both studies, the fibers were significantly larger in the males that displayed the most (top 18%) compared to those that displayed the least (lowest 18%). However, the correlation may not have been detected in the field sample due to the complexity of social interactions or other factors that differed between the field and lab.

The composition of fiber types in the ceratohyoid muscle is similar in males and females, with a majority of fast-twitch fibers (both glycolytic and oxidative-glycolytic). However, the percentage of tonic fibers is increased in males, whereas slow-oxidative fibers are enhanced in females (Rosen et al., 2004). These results are consistent with the idea that the basic features of dewlap extension are similar in the two sexes (Jenssen et al., 2000), but increased tonic fibers may help males to stabilize or maintain the extension of their larger dewlaps.

In green anoles, sex differences in the neuromuscular system controlling hemipenis function during copulation are even greater than in the structures regulating dewlap extension (Ruiz and Wade, 2002). Movement of each hemipenis is controlled ipsilaterally. The transversus penis (TPN) muscle wraps around the ventral surface of the hemipenis and regulates extension through the cloaca. The retractor penis magnus (RPM) muscle is attached to the caudal end of the hemipenis as it lies in the tail and controls its retraction following copulation (Arnold, 1984). Motoneurons innervating both of these muscles are located in the last trunk and first sacral segments of the spinal cord (T17-S1; trunk segments are equivalent to the combined thoracic and lumbar regions of mammals). Within this region of the cord, just over half of the motoneurons project to the TPN and RPM, while most of the rest innervate the caudifemoralis (a muscle that lies mostly in the tail and controls leg movement) and the sphincter cloacae. Hemipenes and the associated muscles are not present in adult females (Ruiz and Wade, 2002). T17-S1 motoneurons are larger and more numerous in adult males than in females (Holmes and Wade, 2004a; Ruiz and Wade, 2002).

**Adult plasticity**

The neuromuscular system regulating dewlap extension is relatively stable in adulthood. That is, morphological characteristics of the ceratohyoid muscle and the motoneurons that innervate it are generally equivalent across the breeding and non-breeding seasons, as well as between individuals in which adult T was manipulated (Neal and Wade, 2007b; O’Bryant and Wade, 2002a). Adult T does appear to influence overall dewlap size, however. In the brown anole (A. sagrei), this treatment increases dewlap area measured from photographs following manual extension (Cox et al., 2009), a measure that likely reflects the length of the second ceratobranchial cartilage. In contrast, exogenous T induces dramatic changes in the size of both hemipenes and RPM fibers, but only in animals exposed to breeding environmental conditions (Holmes and Wade, 2004b). Cross-sectional areas of these structures do not differ between the breeding and non-breeding seasons in unmanipulated animals, perhaps because circulating T does not differ as much between those groups as castrated males given T- or control implants. Regardless, the results from these studies clearly indicate differential levels of responsiveness to T in the courtship and copulatory neuromuscular systems, as well as differential sensitivity of the copulatory system to the same dose of T between seasons.

The expression of AR parallels these morphological differences. AR immunoreactive nuclei are widely present in both the ceratohyoid and RPM muscles, and both the dewlap cartilage and hemipenes express the protein. Most copulatory motoneurons are AR-positive, but the protein is detected in relatively few of those likely to project to the dewlap muscle (Holmes and Wade, 2005b; Rosen et al., 2002). While immunoreactivity did not vary across season in intact males, T increased the percentage of AR-positive nuclei in the RPM of both breeding and non-breeding males, but did not affect it in the ceratohyoid muscle or copulatory motoneurons (Holmes and Wade, 2005b).

A different pattern was detected for changes in muscle fiber type, such that the ceratohyoid exhibited changes in adulthood that were not seen in the RPM. In the dewlap muscle, fiber type composition varied such that proportionally more fast oxidative-glycolytic fibers were detected in intact males during the breeding compared to non-breeding season as well as in T-treated compared to control, gonadectomized males. In the RPM, in which the vast majority of the fibers appear to be of the slow-oxidative type, little if any change due to season or T-treatment was detected (Holmes et al., 2007). These results indicate the potential for T to influence different characteristics of muscles fibers important for behavioral displays — it may modulate dewlap extension by changing biochemical properties and hemipenis function by increasing morphology and androgen sensitivity.

**Developmental organization**

While steroid hormones are likely involved in masculinization of structures in both the dewlap and copulatory systems, the processes differ substantially. The degree and timing of sexual differentiation are distinct between these two neuromuscular circuits. The peripheral structures controlling dewlap extension differentiate far later than those involved in copulation. The dewlap cartilage becomes longer in males than in females between one and two months after hatching, and the fibers of the muscles regulating the display are increased in size in males by post-hatching day 75. The motoneurons differentiate in size sometime between 3 months of age and adulthood (O’Bryant and Wade, 2001). While it does not affect the motoneurons, juvenile T increases (masculinizes) both the length of the cartilage and cross-sectional area of the ceratohyoid muscle fibers (Lovren et al., 2004a).

Hemipenes and associated muscles are present in green anoles of both sexes early in embryonic development, but completely regress in females prior to hatching (Holmes and Wade, 2005a). Interestingly, in leopard geckos the structures are still present in adult females. They regress almost completely, but do not disappear, and grow in response to adult T-treatment (Holmes et al., 2005). T cannot rescue hemipenes or muscles even in juvenile green anole females (Lovren et al., 2004a). It is not clear why this difference is present between the species, but one intriguing possibility relates to the mode of sex determination. Leopard geckos have no sex chromosomes (see earlier), whereas species in the anole genus use an XX/XY system like mammals (Gorman, 1973). It is intriguing to speculate that animals without sex chromosomes maintain more reproductive flexibility, but many more species need to be investigated to adequately address this idea.

Steroid hormones regulate masculine vs. feminine differentiation of the copulatory system. In fact, both appear to be active processes, with embryonic estradiol causing regression and androgen survival/enhancement of both hemipenes and muscles (Holmes and Wade, 2005a). Steroid receptor expression is consistent with this pattern. Both AR and ERα mRNAs are in the hemipenes and muscles of embryonic males and females. At E13 (which is during the period when the structures are regressing in females; Holmes and Wade, 2005a), males express more AR than females, and a trend exists for females to express more ERα (Beck and Wade, 2008b). Interestingly, androgen also increases the size of motoneurons measured on the day of hatching in the region of the spinal cord containing those that control copulatory muscles. However, unlike the hemipenes and muscles, the size and number of these cells appears similar in the two sexes at this time (Holmes and Wade, 2005a) as well as at 90 days.
post-hatching (Lovern et al., 2004a). This monomorphism suggests a substantial dissociation in the timing of sexual differentiation of the peripheral and central structures regulating copulatory behavior.

Conclusions

Arousal and the associated display of motivated behaviors are regulated by a variety of factors in lizards. Adult steroid hormones play prominent roles, but also important are particular stimuli, including environmental cues unique to the breeding season and the presence of male or female conspecifics. Genes and early experiences (exposure to particular incubation conditions and/or steroid hormone levels) can also modulate adult function. These reptilian species provide a rich opportunity for understanding the mechanisms underlying behaviors related to reproduction. An even greater level of knowledge will come from appreciating this information in comparison to other vertebrate groups and in the context of evolutionary history.

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