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Courtship and copulation in the adult male green anole: Effects of season, hormone and female contact on reproductive behavior and morphology

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Abstract

Interactions among reproductive season, testosterone (T) and female presence were investigated on the structure and function of forebrain and neuromuscular systems controlling courtship and copulation in the green anole lizard. Under breeding (BS) or non-breeding (NBS) environmental conditions, male green anoles were implanted with either T or blank capsules and exposed to one of three female stimulus conditions: physical, visual or no female contact. T and at least visual exposure to females increased courtship displays (extension of a throat fan, or dewlap), and these effects were greater during the BS than NBS. T also facilitated copulation, and did so to a greater extent in the BS. The hormone increased soma size in the preoptic area (POA) and amygdala (AMY), and in the AMY the effects were greater in the BS than NBS. Cross-sectional areas of copulatory organs and associated muscle fibers were enhanced by T, and more so in the BS than NBS. However, no effects on morphology of dewlap motoneurons or muscles or copulatory motoneurons were detected. Thus, (1) changes in behavior and neural and/or muscular morphology are not always parallel and (2) differences in responsiveness to T exist across seasons and among tissues.

Keywords

Sexual behavior; Courtship; Lizard; Reptile; Forebrain; Neuromuscular

1. Introduction

Behavioral plasticity is crucial for an animal to adapt to changing environmental influences, and it is frequently accompanied by modifications in the morphology of underlying structures. These types of effects are often seen in reproductive systems of seasonally breeding vertebrates [3,4,17,38,44,53,66,83]. Sexual behaviors can include courtship exhibited by males, such as vocalizations or visual displays, and copulatory behaviors that in some cases involve intromission of a penis by the male [43,87]. While the specific behaviors vary widely across species, testosterone (T) and/or its metabolites estradiol and dihydrotestosterone are vital to the expression of male sexual behaviors across many mammals, birds and reptiles [23,25,55, 63,70,76,77,95,96].

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The expression of male sexual behavior requires the coordination of forebrain regions and neuromuscular circuits [15]. In diverse vertebrates, the preoptic area (POA) and amygdala (AMY) are necessary for the production of male-specific courtship and copulatory behaviors [7,26,43,57,62,93,96,97]. Motoneurons located in the brainstem or spinal cord innervate muscles required for courtship and copulatory behaviors. For example, muscles surrounding the syrinx in songbirds [86], swim bladder in some fishes [6], and larynx in frogs [50] are innervated by caudal brainstem motor nuclei and facilitate vocalizations used by males to court females. Similarly, mammalian copulation involves muscles surrounding the base of the penis that are innervated by motoneurons in the caudal spinal cord [13]. Many of these structures exhibit plasticity that parallel seasonal or T-induced changes in behavior [10,12,14,20,49,62, 65,82,83,92], and they have provided valuable insights about relationships between behavior and morphology. However, full appreciation for key mechanisms may be diminished by studying courtship and copulatory neuromuscular circuits in separate vertebrate groups. To some extent this division exists for good reason; most frogs, fish and birds (whose courtship systems have been exceptional models) do not have penises, and mammals (whose copulatory systems have been extensively studied) do not usually have courtship displays amenable to investigation. It is also rare that forebrain and neuromuscular morphology are investigated in the same experiments.

Green anole lizards (*Anolis carolinensis*) offer a unique opportunity to investigate multiple levels of the nervous system required to coordinate reproduction in the same individuals. This species breeds seasonally, and males exhibit a characteristic suite of sexual behaviors that has been extensively studied in the field and lab [26]. In a courtship encounter, a male anole bobs his head and extends a bright red throat fan, known as a dewlap. If courtship is successful, the male will copulate with the female by intromitting one of two bilateral hemipenes, which normally lay inside the ventral portion of the tail. In unmanipulated animals, dewlap extensions occur far more often in the breeding (BS) than non-breeding (NBS) season, and copulation only occurs during the BS [34,46]. A seasonal increase in T, rather than its metabolites, is the primary regulator of male sexual behavior [28,53,54,74,94].

As in other vertebrates, the POA and AMY (its ventromedial nucleus) facilitate male sexual behaviors in green anoles [35,56]. The neuromuscular components required for courtship include a bilateral pair of muscles located in the throat, the ceratohyoids, which cause the medially located ceratobranchial cartilages to bow out and unfold the dewlap [30]. These muscles are innervated by motoneurons in the caudal brainstem in the vagal component of nucleus ambiguus (AmbX) and the area containing the glossopharyngeal portion of nucleus ambiguus and the ventral motor nucleus of the facial nerve (AmbIX/VII_{mv}) [29,87,88]. To copulate, males evert one of their hemipenes through the cloaca by contracting the transversus penis muscle (TPN) that wraps over it medially to laterally. They retract the hemipene by contracting the retractor penis magnus muscle (RPM) which attaches to the caudal end of the organ as it lies inside the tail. The motoneurons innervating the muscles controlling penis movement are located in the last trunk and first sacral segments (T17–S1) of the spinal cord [40,75,87].

Previous studies of the anole POA and AMY and the courtship and copulatory neuromuscular structures have revealed differences in plasticity in regard to T and season. In intact animals, POA and AMY soma sizes are smaller during the NBS than BS, and T administration during the NBS has no effect on this measure [60]. Although courtship behavior dramatically decreases during the NBS compared to the BS and in castrated compared to T-treated males, the size of dewlap muscle fibers and motoneuron somas appear to remain stable across season and with T manipulations [61]. In contrast, T enhances copulatory muscle, but not motoneuron, morphology during the BS [41]. These separate studies have suggested differences in responses to seasonal and hormonal manipulations, but drawing firm conclusions about relative degrees

of plasticity is difficult because the multiple levels of the nervous system have not been concurrently examined in the same animals [41,61]. Importantly, males in these experiments had no access to females, so potential effects of use and/or social stimuli on reproductive morphology were not addressed. The present experiment was therefore designed to investigate morphological differences in forebrain regions and the courtship and copulatory neuromuscular systems of male green anoles across hormonal and environmental conditions, which included both abiotic and social cues. Males were exposed to T or vehicle control under either BS or NBS temperatures and photoperiods, as well as to one of three levels of contact with females.

2. Materials and methods

2.1. Animals and housing

Experimental adult male and stimulus female green anoles, captured from the field, were purchased during the summer BS and fall NBS from Charles Sullivan Co. (Nashville, TN). Individuals of the two sexes arrived simultaneously in the lab. Females were group housed in 29 gal aquaria, and males were individually housed in 10 gal aquaria. Each aquarium contained sphagnum peat moss bedding, wooden dowels for perching, a water dish and basking rocks. Fluorescent, full-spectrum and incandescent lights simulated natural environmental conditions. BS animals were exposed to long days (14 h of light) with room temperatures ranging from 28 °C during the day to 19 °C at night. NBS animals were exposed to short days (10 h of light) and temperatures varying from 24 °C during the day to 15 °C at night. Incandescent spotlights placed on top of each cage provided animals with basking temperatures up to 10 °C warmer than ambient. Humidity was consistently set at 70%, and aquaria were sprayed daily with water. Animals were fed crickets or mealworms three times a week during the BS and twice each week during the NBS. Procedures were performed in accordance with Michigan State University Institutional Animal Care and Use Committee and NIH guidelines.

2.2. Endocrine manipulations

Following isoflurane anesthesia, males from the BS and NBS were bilaterally gonadectomized while on ice and subcutaneously implanted with a Silastic capsule (7 mm long × 0.7 mm ID × 1.65 mm OD) containing 5 mm of packed testosterone propionate (T) or left blank (BL) (as in [41,42]). Each male was weighed and measured snout-to-vent (nose tip to cloaca; SVL). To facilitate equivalent levels of receptivity among stimulus females, they were ovariectomized and implanted with 5 mm capsules made from a slurry of sealant and 2 mg estradiol benzoate that had been extruded through the tip of a 10 cc syringe (without a needle).

2.3. Behavior testing

Two weeks following surgery, males began a series of 15 min behavior tests administered once on each of 14 consecutive days. Males were randomly assigned to one of three female exposure conditions: (A) a different stimulus female was placed in each male's cage each day; (B) a glass aquarium containing a stimulus female was put immediately next to his; or (C) an empty cage was placed next to it. The number of dewlap extensions and copulations were recorded by an observer blind to hormone manipulation.

2.4. Tissue collection

One day following behavior testing, males were overdosed with sodium pentobarbital, and SVL and body weight were recorded a second time. Males were intracardially perfused with phosphate buffered saline followed by 10% phosphate buffered formalin. Evidence of the Silastic capsule's presence was confirmed at this time. Immediately following perfusion, the brain was removed and post-fixed in phosphate buffered formalin for 7 days. Segments T15–

S2 of the spinal column, the ventral portion of the rostral tail containing the hemipenes and RPM muscles, the throat including ceratohyoid muscles, and the kidneys were collected in Bouin's fixative for 7 days. All tissues were soaked in 70% ethanol, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin. Brains and spinal cords were sectioned at 20 μm . Tails, throats and kidneys were sectioned at 10 μm . Tails and throats were stained with the trichrome method [41], brains and spinal cords were stained with thionin, and kidneys were stained with hematoxylin and eosin.

2.5. Morphological measures

Using Scion (NIH) Image software, soma sizes of 20 randomly chosen neurons in the POA and AMY (ventromedial nucleus), 15 in AmbIX/VIIImv, 10 in AmbX, and 20 in T17–S1 were measured on each side of each animal (as in [41,60,61]). Cross-sectional area was calculated from 25 ceratohyoid and 25 RPM fibers per side [41,61]. An average for each individual was calculated for each of these variables to be used in statistical analyses. Cross-sectional areas of TPN fibers were not determined because they run perpendicular to the RPM and thus cannot be measured in this preparation. An estimate of relative hemipene size was calculated by measuring 10 cross-sections, 50 μm apart [41]. Finally, the height of kidney epithelial cells in renal "sex segments" provides a bioassay for androgen exposure [41,94], so the height of four such cells was measured in each of 10 tubules randomly selected from the two kidneys. A mean from these 40 measurements in each individual was used for statistical comparisons.

2.6. Statistical analysis

The sample sizes for all groups was 8, except for males treated with B1 capsules in the BS who were exposed to empty cages; one individual died during behavior testing ($n = 7$). The total number of dewlap extensions was analyzed with a three-way ANOVA (hormone $\times$ season $\times$ female exposure condition). A two-way ANOVA (hormone $\times$ season) was used for copulatory behavior because only males with a female present in the cage could copulate.

Average soma size for the POA, AMY, AmbIX/VIIImv, AmbX and T17–S1, as well as hemipene, ceratohyoid and RPM muscle fiber size, and kidney epithelial cell height was calculated for each animal, and individually analyzed with three-way ANOVAs (hormone $\times$ season $\times$ female exposure condition). For SVL and body weight, means of measurements recorded at the time of treatment and those taken just before males were perfused were used in three-way ANOVAs. Additional ANOVAs were performed to break down interactions when they existed, and Tukey–Kramer post hoc tests were used for pairwise comparisons. Finally, some regression analyses and analyses of covariance were conducted as appropriate (details in Section 3).

3. Results

3.1. Behavior

Season, T and female exposure all had significant main effects on dewlap extension (Fig. 1A). The behavior was increased in animals tested under BS compared to NBS conditions ($F = 21.20, p < .0001$), with T compared to B1 treatment ($F = 49.30, p < .0001$), and by at least visual exposure to females ($F = 48.46, p < .0001$; Tukey–Kramer, control versus visual or physical contact, both $p < .05$). Significant interactions also existed, such that the effects of T ($F = 5.82, p = .0180$) and female exposure ($F = 3.90, p = .0240$) were greater under conditions typical of the BS than NBS. The effects of T were also greater in males presented with females than control males (treatment $\times$ female exposure condition interaction: $F = 10.12, p < .0001$).

Only males with physical access to females had the opportunity to copulate. For these animals, main effects of season and treatment existed (Fig. 1B) such that only T-treated males ($F =$

49.47, $p < .0001$) copulated, and animals tested during the BS did so more frequently than those in the NBS ($F = 25.94$, $p < .0001$). T-treatment had a greater effect under conditions typical of the BS than NBS ($F = 25.94$, $p < .0001$).

3.2. Morphology

3.2.1. General measures—Although males were randomly selected from shipments, those that arrived in the NBS were on average 16% heavier ($F = 20.70$, $p < .0001$; Table 1) and 3% longer than those from the BS ($F = 8.07$, $p = .0057$; Table 1). No effects of T or female exposure existed on body weight or SVL. Renal sex segments were larger in males tested under conditions typical of the BS than NBS ($F = 37.23$, $p < .0001$; Table 2) and in T- compared to BI-treated males ($F = 1352.09$, $p < .0001$). An interaction also existed, such that the effect of T was greater on animals tested in the BS than NBS ($F = 32.73$, $p < .0001$; Table 2).

3.2.2. POA and AMY—POA soma size was larger in T-treated animals than controls ($F = 6.27$, $p = .014$) and in those tested under conditions typical of the NBS compared to BS ($F = 6.34$, $p = .014$; Figs. 2A and 3). T also increased AMY soma size ($F = 5.59$, $p = .021$), and a season $\times$ T interaction existed in this region ($F = 4.98$, $p = .029$) such that the effect of T was greater in animals tested during the BS than NBS (Figs. 2B and 3). No significant effects of female exposure existed on POA ($F = 0.03$, $p = .974$) or AMY ($F = 1.04$, $p = .360$) soma sizes. Like POA soma size, body weight and SVL were also greater in the NBS than BS (see above). Therefore, regression analyses were performed to determine whether a relationship between body size and POA soma size existed. Across all individuals, both body weight ($R^2 = .09$, $p = .005$) and SVL ($R^2 = .08$, $p = .009$) were positively correlated with POA soma size (data not shown). When potential effects of body weight and SVL on POA soma size were factored out by ANCOVA, the effects of season on POA soma size were eliminated (both $F \leq 2.96$, $p \geq .089$).

3.2.3. Courtship neuromuscular system—No significant effects of T or female exposure existed on ceratohyoid muscle fiber cross-sectional area (all $F \leq 3.53$, $p \geq .063$). However, ceratohyoid muscle fiber size ($F = 7.31$, $p = .0083$) and AmbIX/VIIImv ($F = 21.59$, $p < .0001$) and AmbX ($F = 24.83$, $p < .0001$) motoneuron soma sizes were larger in animals tested under conditions typical of the NBS than BS. As for the POA, these variables were each positively correlated with body weight (ceratohyoid: $R^2 = .17$, $p < .0001$; AmbIX/VIIImv: $R^2 = .06$, $p = .013$; AmbX: $R^2 = .08$, $p = .0075$) and SVL (ceratohyoid muscle: $R^2 = .11$, $p = .0013$; AmbIX/VIIImv: $R^2 = .06$, $p = .019$; AmbX: $R^2 = .05$, $p = .040$). The effects of season on the muscle and both brainstem motonuclei were eliminated if either body weight or SVL were factored out by ANCOVA (all $F \leq 1.69$, and $p \geq .196$).

3.2.4. Copulatory neuromuscular system—T increased the cross-sectional areas of hemipenis ($F = 116.23$, $p < .0001$; Fig. 4A) and RPM muscle fiber ($F = 46.01$, $p < .0001$; Figs. 4B and 5). The effects of T were greater under conditions typical of the BS than NBS on both the hemipenis (interaction: $F = 12.34$, $p = .0007$) and RPM ($F = 10.18$, $p = .0020$; Fig. 4). No main effects of season or female exposure were detected for the hemipenis or RPM (all $F \leq 1.51$, $p \geq .227$). No significant main effects or interactions existed for T17–S1 motoneuron soma size (all $F \leq 3.05$, $p \geq .085$; data not shown).

4. Discussion

4.1. Summary and comparison to previous anole work

The present results demonstrate that male sexual behavior in the green anole is facilitated by each of the three factors investigated: T, environmental conditions typical of the BS, and exposure to females. Interactions among these variables also indicate that the display of male

sexual behaviors is more responsive to the effects of T and the presence of females during the BS than NBS, and to females when T levels are relatively high. Perhaps the most interesting results, however, involve the varied influences of hormone and seasonal conditions on the morphology of the different tissues. The peripheral copulatory system (hemipenes and RPM muscle fibers) substantially increased in size with T-treatment, whereas in the same individuals fibers of the muscle responsible for dewlap extension did not. T also appeared to induce growth in POA and AMY soma size, but not in the motonuclei responsible for dewlap extension or hemipene movement.

Importantly, parallel to reproductive behaviors, morphology of each of these structures, with the exception of the POA, was more responsive to T under conditions typical of the BS than NBS. The renal sex segments also showed a greater increase in cell size due to T in the BS compared to the NBS. These data suggest that this pattern is not limited to neural and muscular structures, and that reduced responsiveness to T in the NBS may occur throughout androgen-sensitive tissues. While possible, it seems quite unlikely that the greater effects of T during the BS were due to a methodological difficulty, such as less T being released from the capsules in the NBS. Soma size in the POA was increased equivalently by T in the two seasons, which is consistent with the idea of comparable exposure. In addition, while it was not directly quantified, visual inspection suggested that approximately the same amounts of T remained in the capsules of BS and NBS males at the time of perfusion. Finally, while we did not collect plasma to measure T directly, other studies have found equivalent circulating T in the BS and NBS after T capsule implantation [79].

The behavioral and morphological effects of T and season that were detected in this study are consistent with and extend prior work, allowing for more complete conclusions. That is, as in the present study, others have documented that T is important for the display of male sexual behavior in the green anole [53,87]. The present data also replicate T-induced increases in the size of hemipenes, copulatory muscle fibers and renal sex segment cell size, with greater effects under conditions typical of the BS than NBS [41,42,53,61,87]. Fewer statistically significant results were detected in the earlier study on the copulatory system, but the magnitude of the differences was nearly identical to those in the present study. Here, T induced approximately a 124% increase in hemipenis and RPM size in the BS and a 33% increase in the NBS. In Holmes and Wade [41], these values were 129% and 33%, respectively. Thus, it is likely that the approximately three-fold increase in sample size contributed to interactions between season and T detected in the current, but not earlier, study. The lack of specific effects of T and seasonal environmental conditions in castrated males on the copulatory motoneurons and dewlap muscle fibers and motoneurons also replicate previous work [41,61]. Finally, an earlier study from our lab indicated that the soma size of neurons in the POA and AMY is not increased by T when administered under conditions typical of the NBS. However, it did not investigate the effect of the hormone on these cells in the BS. The present data are consistent with those results, particularly for the AMY in which a season $\times$ T-treatment interaction existed. They also indicate that if BS animals are added to the analysis, T does facilitate an increase in the size of cells in these regions. Thus, by evaluating behavior as well as morphology in three portions of the nervous system (limbic forebrain, and dewlap and copulatory neuromuscular) in the same individuals, we have been able to document clear and consistent differences in behavioral and morphological responsiveness to T at multiple levels—across structures, as well as between seasons.

The present data also for the first time consider the specific effects of female exposure. While it is no surprise that at least visual exposure to females is required to stimulate male sexual behavior, the fact that it had no direct effect on the morphology of any structure that was evaluated is quite intriguing. These results suggest that there are limits on the environmental cues that stimulate morphological change (that abiotic rather than social stimuli are relevant),

and perhaps more importantly, the data are consistent with the idea that increases in use do not cause changes in the sizes of these neural or muscular cells. It is possible that a greater range in the total number of dewlap extensions or copulations would be required to detect such an effect, but if a relationship between degree of function and morphology exists, it is likely that it is quite subtle.

The question, of course, is why the behavioral and morphological differences in responsiveness to T exist. On a functional level, morphology of structures important for dewlap extension may need to be maintained because the dewlap is used during the NBS (although far less frequently than the BS), in contrast to copulation which occurs rarely if ever in the NBS [45,53,60]. On the other hand, T's increase of hemipenis size during the BS might serve a number of purposes, including facilitation of sperm delivery. And, a larger hemipene might require larger RPM muscle fibers. Increases in soma size in the POA and AMY in response to T may have less to do with "mechanics" and instead reflect the changes in function associated with integration of environmental cues and the hormonal status of a male to determine whether mating is appropriate [80].

T-induced changes in morphology and behavior may involve androgen receptor (AR) expression. ARs are expressed in the anole POA and AMY during the BS [72], but further work is needed to determine whether AR expression in these areas is modified by either T or environmental conditions. We do have information about neuromuscular structures, however. ARs are located throughout the copulatory system, and exogenous T increases their expression in the hemipenes and RPM, although it does not increase the percent of AR-positive T17-S1 motoneurons [42]. ARs are also expressed in ceratohyoid muscles and 45% of AmbX motoneurons, but apparently not in AmbIX/VIIImv motoneurons in intact males [42,72]. Unlike the copulatory system, but consistent with the relative lack of plasticity, T does not seem to alter AR expression in dewlap structures [42]. Therefore, T may enhance morphology of peripheral copulatory, but not courtship, structures at least in part via an up-regulation of AR.

The hormone may also influence morphology through other mechanisms. For example, aromatase activity is greater in whole brain homogenates from breeding compared to non-breeding adult male green anoles [73]. Aromatase activity (estradiol) is not required for the display of masculine behavior in this species [94]; T itself is the most potent activator of male sexual behavior [87]. However, the effect of estrogen on brain morphology has not been investigated in this species. It is therefore possible that T's increase of POA and AMY soma size is mediated by estrogen receptors and/or an increase in aromatase activity, although at this point we do not know whether neural aromatase is increased by T in green anoles. Likewise, potential roles of other steroid-related factors that may change seasonally, such as steroid binding globulins and receptor co-activators have not yet been explored.

4.2. Reproductive behavior and morphology in other species

Like the anole, behavioral and morphological plasticity exist in other vertebrate species. For example, some seasonally breeding male songbirds sing to court females and copulate with them during the BS when T levels are high, and these behaviors diminish during the NBS when T levels decline [3,66]. In male starlings, T increases the volume of song control nuclei more under conditions typical of the BS than NBS [8]. Parallel to behavioral changes, the volume of brain regions controlling song can increase due to the rise in T that occurs in the BS [3,4,16,19,59,69,81,83,84]. However, unlike the anole, increased song production in some birds contributes directly to enhancement of the morphology of song control nuclei [1,18,85].

T and/or breeding environmental conditions enhance male sexual behavior and in parallel enhance volume and/or soma size of the POA and AMY in Japanese quail, whiptail lizards and rodents [22,24,25,27,62,71,82,87,90,91]. Interestingly, as in the present study, a seasonal

environmental cue (photoperiod) influences the response of a portion of the AMY (the MeApd) to T in Siberian hamsters. Soma size in and volume of this brain region are increased by treatment with this hormone to a greater extent in males housed in long- than short-days. These data parallel those indicating that photoperiod mediates the ability of T to regulate gonadotropin secretion, neuropeptide immunostaining, opiate binding and androgen receptor expression in particular brain regions in these animals, as well as those documenting that androgen fails to restore mating behavior in Syrian hamsters housed on short days [9,23].

Exogenous T, as well as naturally occurring seasonal increases in T, also induce masculine reproductive behaviors and enhance muscle and/or motoneuron size in courtship structures in some birds, frogs, fish and copulatory muscle and/or motoneuron size in mammals [2,5,12–14,21,31–33,36,37,39,48,50,51,64,65,67,89,92]. However, morphology and behavior do not respond to T and seasonal environmental conditions in parallel in all cases. For example, in free-living male song sparrows the volume and soma size of song control regions differ between the BS and NBS, but song repertoire size does not [78]. In wild canaries, seasonal rises in T and learning of new song syllables do not result in morphological changes to fore-brain song production areas [52], but in a captive population of canaries song production areas show seasonal increases in volume parallel to increases in song learning [58]. In tree lizards, seasonal fluctuations in the volumes of the POA and AMY exist, but hormones appear to play little, if any, role. And, levels of aggressive behavior (measured in this study rather than reproduction) are not associated with these differences [47]. Other examples of differential effects on morphology and function include facilitation of masculine sexual behaviors by T in whip-tail lizards of both sexes, but T increases POA volume only in males [91]. Similarly, larger mean muscle mass and motoneuron soma size are associated with *decreased* copulatory behavior in male rats [11,68].

5. Conclusions

The POA and AMY, plus the dewlap and copulatory neuromuscular structures are components of a circuit necessary for the display of the full suite of reproductive behaviors in the male green anole. However, differences in the degree of plasticity exist across these levels; they respond uniquely to seasonal and hormonal cues. Investigating multiple levels of this reproductive system has provided an integrated picture of relationships among T, morphology and behavioral function in green anoles, including seasonal and structural differences in the sensitivity to T. In some cases, these effects parallel those in other vertebrate groups. However, critical differences exist, perhaps including a relatively unique change in the structural and functional responses of tissues to T across seasons. To our knowledge, the issue of differences in responsiveness to T across seasons, particularly the variation among tissues responsible for coordinating different levels of reproductive behaviors, has not been considered fully in other model systems. It will now be important to investigate in depth the mechanisms behind the potentially differential sensitivity to T, both across structures and seasons, in particular because the most obvious mediator (level of AR expression) does not appear directly responsible.

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Fig. 1.

Total number of dewlap extensions (A) and copulations (B) under environmental conditions typical of the breeding and non-breeding seasons in gonadectomized males implanted with either testosterone (T) or blank (BI) capsules, and exposed to one of three levels of female contact. Statistically significant main effects of season, hormone treatment and female exposure condition, as well as interactions among season and hormone treatment, season and female exposure, and hormone treatment and female exposure were detected for total dewlap extensions. Main effects of season and hormone treatment and an interaction between season and hormone treatment existed in males with female contact for total copulations.

Fig. 2. Soma size in the preoptic area (POA) (A) and amygdala (AMY) (B) under environmental conditions typical of the breeding and non-breeding seasons in gonadectomized males implanted with either testosterone (T) or blank (Bl) capsules, and exposed to one of three levels of female contact. Means $\pm$ S.E. are depicted.

Fig. 3.
Photomicrographs of neurons in the preoptic area (A and B) and amygdala (C and D) taken from breeding conditions males. (A) and (C) are from males treated with T, and (B) and (D) are from males implanted with BI capsules. Scale bar = 10 μ m.

Fig. 4.

Cross-sectional area of the hemipenis (A) and retractor penis magnus (RPM) fibers (B) under environmental conditions typical of the breeding and non-breeding seasons in gonadectomized males implanted with either testosterone (T) or blank (BI) capsules, and exposed to one of three levels of female contact. Mean values $\pm$ S.E. are depicted for each group. Main effects of season and an interaction between season and hormone treatment existed for both hemipenis (A) and RPM fiber size (B).

Fig. 5. Photomicrographs of cross-sections through RPM muscle fibers under environmental conditions typical of the breeding (A and B) and non-breeding (C and D) seasons. (A) and (C) are from T-treated, and (B) and (D) from BI-treated males. Scale bar = 100 μ m.

Table 1

Mean $\pm$ S.E.M. body weight and snout-to-vent length (SVL) during the breeding (BS) and non-breeding (NBS) seasons

	BS*	NBS
Body weight (g)	4.40 $\pm$ 0.07	5.09 $\pm$ 0.12
SVL (mm)	61.08 $\pm$ 0.33	63.00 $\pm$ 0.56

* Significant effect of season. Values represent averages across hormone treatment and female exposure conditions because these variables had no effect on body weight or SVL.

Table 2

Mean $\pm$ S.E.M. renal sex segment cell height in testosterone (T) and blank (Bl)-treated males during the breeding (BS) and non-breeding (NBS) seasons

	BS*	NBS
T [#]	46.60 $\pm$ 1.12	37.20 $\pm$ 0.99
Bl	12.87 $\pm$ 0.29	12.52 $\pm$ 0.26

* Significant effect of season;

[#] significant effect of T; an interaction between these manipulations was also detected. Measurements are averaged across female exposure conditions because this variable did not affect cell height.