Species Differences in the Regulation of Tyrosine Hydroxylase in *Cnemidophorus* Whiptail Lizards

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ABSTRACT: Evolution of behavioral phenotype involves changes in the underlying neural substrates. *Cnemidophorus* whiptail lizards enable the study of behavioral and neural evolution because ancestral species involved in producing unisexual, hybrid species still exist. Catecholaminergic systems modulate the expression of social behaviors in a number of vertebrates, including whiptails, and therefore we investigated how changes in catecholamine production correlated with evolutionary changes in behavioral phenotype by measuring the size and number of catecholamine producing (tyrosine hydroxylase-immunoreactive, or TH-ir) cells across the reproductive cycle in females from two related whiptail species. *Cnemidophorus uniparens* is a triploid, parthenogenetic species that arose from hybridization events involving the diploid, sexual species *C. inornatus*. Prior to ovulation, females from both species display female-like receptive behaviors. However, after ovulation, only parthenogenetic individuals display malelike mounting behavior. In all nuclei measured, we found larger TH-ir cells in the parthenogen, a difference consistent with species differences in ploidy. In contrast, species differences in the number of TH-ir cells were nucleus specific. In the preoptic area and anterior hypothalamus, parthenogens had fewer TH-ir cells than females of the sexual species. Reproductive state only affected TH-ir cell number in the substantia nigra *pars compacta* (SNpc), and *C. uniparens* individuals had more TH-ir cells after ovulation than when previtellogenic. Thus, species differences over the reproductive cycle in the SNpc are correlated with species differences in behavior, and it appears that the process of speciation may have produced a novel neural and behavioral phenotype in the parthenogen.
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INTRODUCTION

Neural and behavioral evolution are often difficult to study because ancestral species no longer exist. *Cnemidophorus* lizards enable the study of evolutionary processes because new species arise through multiple hybridizations. *Cnemidophorus uniparens*, for example, is a triploid parthenogen that arose through two hybridization events, both involving the diploid, sexual species *C. inornatus* (Wright, 1993). Though females of both species show identical patterns of steroid hormone secretion across the reproductive cycle, there are considerable behavioral differences between the two. Females of both species display female-like receptive behaviors during vitellogenesis when estrogen levels are rising. However, following ovulation when there is a surge of progesterone, females of the ancestral, sexual species become sexually unreceptive while the parthenogens display male-like copulatory behaviors (Moore and Crews, 1986; Moore et al., 1985a,b; reviewed in Crews and Sakata, 2000). By investigating the neural correlates of this behavioral

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difference, we can elucidate the mechanism underlying the evolution of this behavior.

There is considerable homology in the distribution of catecholaminergic systems across vertebrates (Smeets and Gonzalez, 2000) and there may be similarity in the function of those systems, as catecholamines modulate reproductive behaviors in a number of vertebrate species. For example, dopamine agonists affect the display of sexual behaviors in mammals (Melis and Argiolas, 1995) and birds (Absil et al., 1994; Balthazart et al., 1997) and increase the display of male-like mounting behavior in *C. inornatus* males and in *C. uniparens* individuals (Woolley et al., 2001). Further, dopamine release into the striatum, nucleus accumbens, and limbic nuclei increases during copulation in rats (Damsma et al., 1992; Hull et al., 1995; Pfaus et al., 1990). Catecholamine systems are also steroid sensitive, with steroid hormones affecting transmitter synthesis and release (Gunnet et al., 1986; Mitchell and Stewart, 1989) as well as the expression of pre- and postsynaptic receptors (Becker, 1999; Hruska and Nowak, 1988; Lammers et al., 1999; Lee and Mouradian, 1999). Thus, one means by which steroid hormones may regulate the display of reproductive behaviors may be through changes in the synthesis, release, or reception of catecholamines. Because *C. uniparens* and *C. inornatus* differ in the regulation of steroid hormone receptors across the reproductive cycle (Godwin and Crews, 2002), species differences in the display of reproductive behaviors could also result from the differential regulation of dopaminergic systems by steroid hormones.

We investigated differences in the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, in limbic and midbrain nuclei across the reproductive cycle in *C. uniparens* and *C. inornatus* under the hypothesis that ploidy and reproductive state would interact to affect the size and number of TH-ir cells. Work on amphibians has found that cell size is correlated with neural complexity in the optic tectum, including cell size and number (Roth et al., 1994). Specifically, species with higher ploidy tend to have larger but fewer cells in the optic tectum. In addition, previous studies on whiptails have found that in the parthenogen, neurons in the preoptic area and ventromedial hypothalamus have larger somas than do neurons in the same nuclei in the ancestral diploid species (Wade and Crews, 1992). Thus, we predicted that there would be larger but fewer TH-ir cells in the triploid parthenogen than in the diploid ancestral species. In addition, we hypothesized that changes in catecholamine synthesis or release may modulate changes in behavior across the reproductive cycle and therefore we predicted that cell number would be affected by reproductive state. We report that while ploidy affected the size of catecholaminergic cells similarly across nuclei, with larger cells in the parthenogen, species differences in the number of cells were nucleus dependent and correlated with species differences in behavioral phenotype.

METHODS

Housing

The individuals used in this study were housed in the laboratory from the summer of 2001 until February of 2002. *C. uniparens* individuals were collected near Portal, Arizona under state permits from Arizona and New Mexico in the summer of 2001. After being brought into the lab at the University of Texas individuals were housed in groups of four to five in 75 × 32 × 32 cm aquaria. *Cnemidophorus inornatus* females were collected outside Sanderson, Texas in the summer of 2001 and three to four females and one male were housed together in 75 × 32 × 32 cm aquaria. During the summer of 2001, all individuals were housed in environmental chambers on a 14:10 L/D light cycle with temperatures fluctuating from 33°C during the day to 23°C during the night. In October 2001, individuals were gradually introduced to conditions resembling hibernation by decreasing photoperiod and temperatures on a weekly basis. During hibernation, individuals were kept on a 8:16 L/D photothermal cycle with temperatures fluctuating from 12.5°C during the day to 10°C at night. After 10 weeks in full hibernation, photoperiod and daily temperatures were gradually increased on a weekly basis until returning to the summer photothermal regime in January of 2002. Individuals were housed at summer photothermal conditions for 4 weeks prior to sacrifice to allow sufficient exposure to summer temperature and light cycles for females to become reproductively active. Individuals received crickets or mealworms dusted with vitamin powder 2–3 times/week during summer conditions and once/week during hibernation and water was provided *ad libitum* year round. Each group cage contained wood blocks for shelter.

Animals

Brains were collected from 10 females in each of three reproductive states: pre- or early vitellogenic, mid- or late vitellogenic, or postovulatory. Reproductive state was determined by palpating the ventral side of each individual and was confirmed after sacrifice. The diameters of vitellogenic follicles were measured after sacrifice. Individuals with follicles less than 2 mm in diameter or no follicles were classified as previtellogenic, individuals with follicles 6 to 10 mm in diameter were classified as vitellogenic, and individuals with eggs were classified as postovulatory. Previous studies using this classification of reproductive state based on follicle size and the presence or absence of eggs

have determined both the level steroid hormones (Moore and Crews, 1986; Moore et al., 1985b) and display of reproductive behaviors (Lindzey and Crews, 1988a; Moore et al., 1985a) that are correlated with each state. The snout-vent length (the distance from the end of the nose to the cloaca) of each individual was also recorded prior to sacrifice. Individuals were anesthetized on ice prior to decapitation and all procedures were performed in accordance with NIH and institutional guidelines on the care and use of animals.

TH Immunohistochemistry

Brains were soaked in 4% paraformaldehyde in phosphate buffered saline for 48 h at 4°C then soaked in 20% sucrose overnight and then frozen in isopentane and stored at −80°C until sectioning. Serial 60 μm sections were cut on a cryostat and two sets of tissue were collected and stored in antifreeze (1% polyvinyl pyrrolidone, 30% sucrose, 30% ethylene glycol in TBS) at −20°C. One set was used for the TH immunohistochemistry described in this study.

Immunohistochemistry was performed on free-floating sections and all brains were run in the same assay. Sections were rinsed overnight in 0.05 *M* Tris buffered saline (TBS; pH 7.7), then incubated in 3% hydrogen peroxide and 4% normal goat serum in TBS for 20 min. After blocking for 1 h in 4% normal goat serum, sections were incubated for 72 h at 4°C in a monoclonal primary antibody (1:600, mouse anti-TH; Chemicon International, Temecula, CA) with 4% goat serum. The antibody has been used in a number of other studies on reptiles and has been demonstrated to react with TH in lizards (Lopez et al., 1992). Sections were then incubated for 2 h in a horseradish peroxidase conjugated goat antimouse secondary antibody (1:350; Bio-Rad, Hercules, CA). Immunoreactivity was visualized using 3,3 diaminobenzedine (DAB; Vector Labs). Sections were then mounted and dehydrated onto slides and counterstained with a Nissl stain (cresyl violet). Sections incubated in 4% goat serum in the absence of primary antibody were used as negative controls and labeled cells were never seen on control sections.

Cell Counting and Analysis

Slides were randomized and coded so that we were blind to species and reproductive state. Sections were imaged using a Zeiss microscope fitted with a Ludl Electronic Products MAC 2002 motorized stage (LEP, NY), an Optronics DEI 750 camera (Optronics, CA), and a Dell Pentium III XPS B733r computer.

We found TH-ir cells in a number of nuclei within the elencephalon, diencephalon and midbrain, and most of the populations we identified appeared similar to those described in other reptiles (reviewed in Smeets, 1994; Smeets and Gonzalez, 2000). The most rostral diencephalic population was located in the preoptic area (POA) with cells located near the ventricle in the periventricular POA and more medially in the POA [Fig. 1(A)]. In the caudal POA we found a number of ventrally located cells in a cell sparse region near the ventricle. Unlike cells in the periventricular POA, these cells were smaller, and many of them appeared to be CSF contacting. This population may be the preoptic recess that has been found in *Anolis carolinensis* (Lopez et al., 1992) as well as the turtle *Trachemys scripta* (Smeets and Steinbusch, 1990). A large population of TH-ir cells was found in the anterior hypothalamus (AH) located just caudal to the POA and anterior commissure [Fig. 1(B)]. In *Cnemidophorus*, the anterior hypothalamus appears as a contiguous group of cells forming an inverted L shape when visualized with Nissl. Although a similar nucleus has been described as being the rostral portion of the nucleus dorsalis hypothalami in another teiid, *Tupinambis nigropunctatus* (Cruce, 1974), we will use the nomenclature established in Young et al. (1994) here to be consistent with other studies on *Cnemidophorus* lizards. The TH-ir cell bodies in the AH were found on the ventrolateral edge of the nucleus. There was a population of TH-ir cells in the dorsal hypothalamus (DH), which is located caudal and dorsal to the AH, and dorsolateral to the periventricular organ [Fig. 1(C)]. This TH-ir cell group appears to be similar to a population originally referred to in amphibians as the "accompanying cell group of the periventricular organ" (p. 466) by Gonzalez and Smeets (1991). We found a population of cells in the medial midbrain that is similar to populations in other reptiles and has been compared to the mammalian ventral tegmental area, and a population of cells that spread from ventromedial to dorsolateral that is thought to be similar to the mammalian substantia nigra pars compacta [SNpc; Fig. 1(D)].

We counted the number of TH-ir cells in the medial and periventricular POA, AH, DH, and SNpc. We did not count the number of cells in the preoptic recess because these cells often overlapped to such a degree that single cells could not be distinguished from each other or the background. Also, because drawing definitive boundaries between the SNpc and VTA was difficult, we only counted cells from the SNpc on sections just caudal to the VTA. Nuclei were delineated based on Nissl staining using camera lucida drawings and descriptions provided in Young and Crews (1995) and the forebrain atlas for *Gekko gecko* (Smeets et al., 1986), and TH-ir cell populations were compared to those for other reptiles reviewed in Smeets (1994) and the references therein.

Cells within each nucleus were counted using StereoInvestigator software (Microbrightfield, VT), which produces a live image of the slide on a computer screen, allowing the user to focus up and down in order to visualize cells throughout the thickness of the section. The area of each nucleus was outlined on each section based on Nissl-defined boundaries using a 4X objective lens. To count the cells, the user identified all TH-ir cells within the nucleus using a 40X objective lens and placed a mark on the image of the cell on the screen. As there was little to no background staining on the sections, cells were considered labeled if they were dark brown relative to the surrounding tissue. For each individual, we counted all cells unilaterally in each nucleus on all

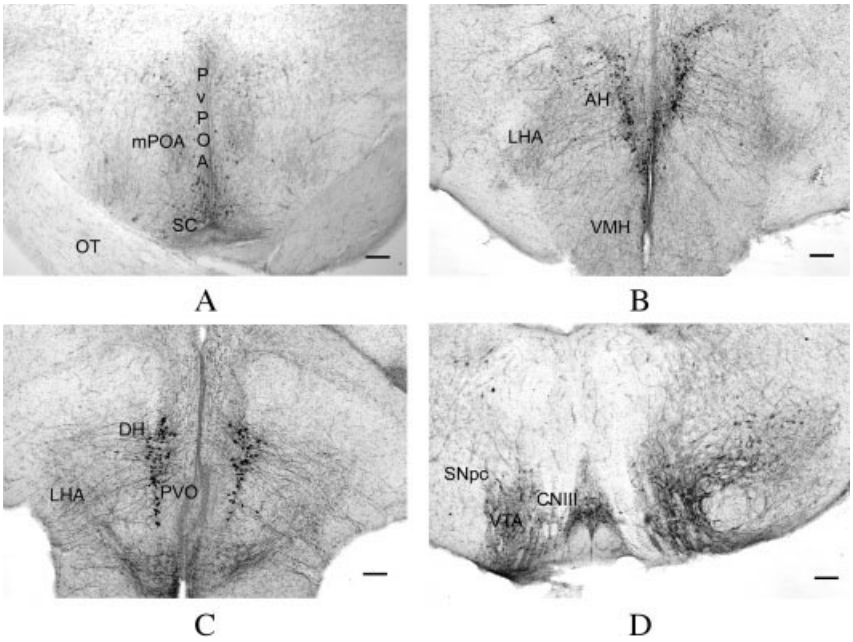

Figure 1 Photomicrographs of TH-ir cells in the (A) preoptic area, (B) anterior hypothalamus, (C) dorsal hypothalamus, and (D) midbrain. Images were taken of sections with reddish-brown DAB stained TH-ir cells and a purple-blue Nissl counterstain. To enhance to appearance of the TH-ir cells, a blue filter was implemented using SPOT imaging software version 3.5.9 (Diagnostic Instruments, MI) during image acquisition and images were converted to grayscale. Consequently, TH-ir cells and fibers appear black while Nissl stained cells appear gray, or, in many cases, are filtered out entirely. Bar indicates 100 μm. AH: anterior hypothalamus; CNIII: cranial nerve three; DH: dorsal hypothalamus; LHA: lateral hypothalamic area; mPOA: medial preoptic area; PvPOA: periventricular preoptic area; PVO: periventricular organ; SC: suprachiasmatic nucleus; SNpc: substantia nigra pars compacta; VMH: ventromedial hypothalamus; VTA: ventral tegmental area.

sections where the nucleus was present. Two to four sections were counted per nucleus per individual, and the number of cells/section was averaged across all sections for each individual. Because there are considerably fewer TH-ir cells present in these nuclei than would be necessary to perform unbiased stereological estimates of the number of cells per nucleus, and because some sections were damaged during processing, we report the mean number of cells per section rather than an estimate of the total number of cells per nucleus.

The somal areas of eight randomly chosen cells on two sections per nucleus per individual (16 cells per nucleus per individual) were measured using a nucleator program (MicroBrightfield). The nucleator program required the user to identify a point near the center of the cell. From that point a set of eight rays was extended, and the intersection of each ray with the boundaries of the cell was marked by the user and the somal area was calculated as the area of the octagon created by connecting all the points along the cell boundary.

Statistical Analysis

We performed one mixed model analysis of variance (ANOVA) for the number of TH-ir cells and one for the size of TH-ir cells, each with species (*C. inornatus* vs. *C. uniparens*), reproductive state (previtellogenic, vitellogenic, postovulatory), and nucleus (POA, AH, DH, and SNpc) as the independent variables. Individual identity was also included as a random variable nested within species and reproductive state; adding this factor eliminates the variability among subjects due to individual differences from the error term (Sokal and Rohlf, 1995; Stevens, 1996). If there was a significant effect of nucleus or reproductive state, we performed posthoc contrasts with an adjusted α based on the number of contrasts (Bonferroni correction for six comparisons for nucleus $\alpha = 0.008$; Bonferroni correction for three comparisons for reproductive state $\alpha = 0.0167$). When there was a significant species by nucleus, reproductive state by nucleus, or species by reproductive state by nucleus interaction we did analyses of each nucleus independently using a two-way ANOVA test with species and reproductive state as independent variables and the mean number of cells per section in each nucleus (POA, AH, DH, and SNpc) as the dependent variables.

For the ANOVAs within each nucleus, when there was a significant effect of reproductive state we performed planned contrasts between the different reproductive states and adjusted our $\alpha = 0.0167$ (Bonferroni correction for

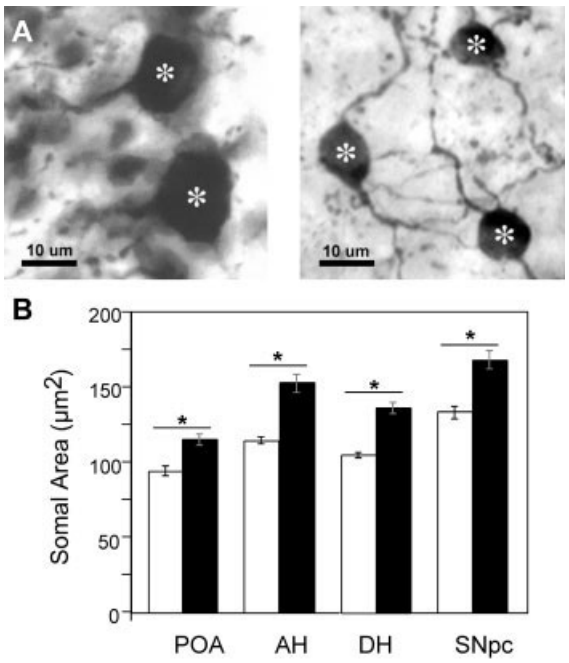

Figure 2 (A) Photomicrographs of TH-ir cells in the DH of *C. uniparens* (left panel) and *C. inornatus* (right panel) illustrating species difference in somal area. TH-ir cells are indicated with white asterisks. (B) Mean somal areas of TH-ir cells in the POA, AH, DH, and SNpc. The TH-ir cells in *C. inornatus* females (white bars) are significantly smaller than those in *C. uniparens* individuals (black bars) in all four nuclei.

three comparisons). When the interaction between species and reproductive state was significant, we performed planned contrasts between reproductive states (previtellogenic vs. vitellogenic, previtellogenic vs. postovulatory, vitellogenic vs. postovulatory) within each species, and between species within each reproductive state (e.g., postovulatory *C. uniparens* vs. postovulatory *C. inornatus*). For the planned contrasts of species by reproductive state interactions we set $\alpha = 0.0055$ (Bonferroni correction for nine comparisons) to control for multiple comparisons. Data were analyzed using JMP version 3.2 statistical software for the Macintosh.

RESULTS

For the analysis of cell size, there was an overall effect of species [$F(1, 97) = 71.29$; $p < 0.001$] where *C. uniparens* had larger cells than *C. inornatus* females (Fig. 2). There was also a significant effect of nucleus [$F(3, 97) = 31.74$; $p < 0.001$]. Posthoc contrasts revealed that cells in the SNpc were significantly larger than those in the AH [$t(3, 97) = -6.23$; $p < 0.001$], the DH [$t(3, 97) = -3.5$; $p = 0.007$], and the POA [$t(3, 97) = -9.27$; $p < 0.001$]. Cells in the AH were significantly larger than those in the DH [$t(3, 97) = -3.116$; $p < 0.0024$] and POA [$t(3, 97) = -3.31$; $p < 0.0013$], and cells in the DH were significantly larger than cells in the POA [$t(3, 97) = -6.40$; $p < 0.001$]. There was no effect of reproductive state or any significant interactions between nucleus, species, or reproductive state. These results are similar to the findings of Wade and Crews (1992) of a species difference in cell size and a lack of modulation of size by reproductive state of Golgi stained cells in the hypothalamus and POA.

In the mixed model ANOVA for the number of TH-ir cells, there was an overall effect of reproductive state [$F(2, 105) = 3.43$; $p = 0.041$]. Posthoc tests indicated that postovulatory and vitellogenic individuals had more TH-ir cells than previtellogenic individuals, though these differences did not reach significance. There was also a significant effect of nucleus where posthoc contrasts found that the AH and SNpc had a similar number of cells, and each had significantly more cells than the DH [for AH $t(2, 105) = 7.46$; $p < 0.001$; for SNpc $t(2, 105) = -5.33$; $p < 0.001$] and POA [for AH $t(2, 105) = 11.44$; $p < 0.001$; for SNpc $t(2, 172) = -9.04$; $p < 0.001$]. There was a significant species by reproductive state interaction [$F(2, 105) = 3.59$; $p = 0.035$]. There was also a significant species by nucleus interaction [$F(2, 105) = 3.55$; $p = 0.017$] and a significant reproductive state by nucleus interaction [$F(2, 105) = 2.38$; $p = 0.034$]. Based on these significant interactions, we performed ANOVAs of each nucleus.

When we analyzed each nucleus individually we found a significant effect of species on the number of cells in the AH [$F(1, 40) = 8.21$; $p = 0.007$; Fig. 3]. *Cnemidophorus inornatus* females had more cells in the AH than *C. uniparens* individuals. *Cnemidophorus inornatus* females also had more cells than *C. uniparens* individuals in the POA, however, this difference only approached significance [$F(1, 36) = 3.67$; $p = 0.063$]. There was no effect of species on the number of cells in the DH. There was no effect of reproductive state, or a reproductive state by species interaction in the AH, POA, or DH.

There was a significant effect of reproductive state on the number of cells in the SNpc [Fig. 4; $F(2, 34) = 4.28$; $p = 0.022$]. Posthoc contrasts revealed that postovulatory animals had numbers of TH-ir cells similar to vitellogenic animals and had significantly more cells than previtellogenic animals [$t(2, 34) = 2.59$; $p = 0.014$]. Vitellogenic individuals also had more cells than previtellogenic individuals, though this effect was not significant. There was also a trend toward an interaction between species and reproduc-

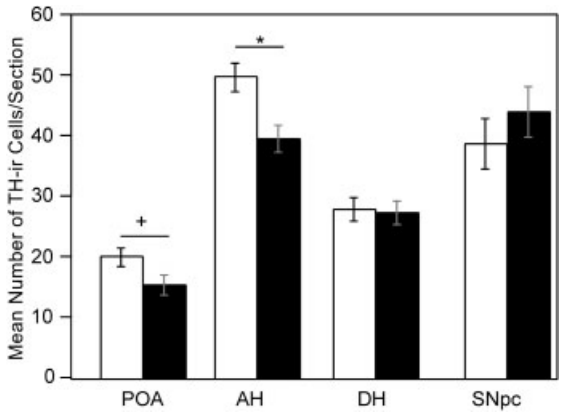

Figure 3 Females of the sexual species, *C. inornatus*, (white bars) have significantly more cells than the parthenogen (black bars) in the AH, and marginally more cells in the POA. There was no difference between the species in the DH or the SNpc, and no effect of reproductive state in the POA, AH, or DH . * indicates a significant difference at $p < 0.05$. + indicates $p < 0.07$.

tive state [$F(2, 34) = 2.55$; $p = 0.093$]. Planned contrasts indicated that postovulatory *C. uniparens* had significantly more TH-ir cells in the SNpc than previtellogenic *C. uniparens* individuals [$F(1, 16) = 12.03$; $p = 0.001$]. There was not a significant effect of species on the number of TH-ir cells in the SNpc.

DISCUSSION

Cnemidophorus uniparens individuals and *C. inornatus* females differ in the display of reproductive behaviors across the ovulatory cycle and we investigated whether the size or number of TH-ir cells in limbic and midbrain nuclei was correlated with differences in ploidy or reproductive state. We found that TH-ir cells were consistently larger in *C. uniparens*, perhaps an effect of the species difference in ploidy, whereas species differences in TH-ir cell number were nucleus dependent. The parthenogen had significantly fewer cells than the sexual species in the AH, and slightly fewer cells in the POA, regardless of reproductive state. In the DH, the species had a similar number of TH-ir cells. In the SNpc, postovulatory *C. uniparens* had a greater number of cells than previtellogenic *C. uniparens*, while there were no differences in the SNpc of *C. inornatus* females across the reproductive cycle. These data indicate that ploidy and changes in gonadal steroid secretions do not have similar effects on the number of TH-ir neurons across different brain areas. Consequently, the process of speciation appears to have differentially affected neurochemical circuits as well as neural morphological parameters. The two species may achieve behavioral differences, and even behavioral similarities, through the effects of differences in cell size and cell number.

The SNpc was the only nucleus to show a significant effect of reproductive state on the number of TH-ir cells, and moreover, the effect of reproductive state was species specific. Postovulatory *C. uniparens* had significantly more cells than previtellogenic *C. uniparens* individuals and more, though not significantly, than postovulatory *C. inornatus* females. *Cnemidophorus uniparens* individuals and *C. inornatus* females differ behaviorally during the postovulatory phase, when *C. uniparens* display male-like mounting behaviors while *C. inornatus* females become sexually unreceptive (reviewed in Crews and Sakata, 2000). While preoptic and hypothalamic nuclei are often implicated in the control of sexual behaviors, recent work indicates that the SNpc is also involved in sexual and motivated behaviors in mammals. In mammals, dopaminergic cells in the SNpc show greater dopamine release into the striatum with locomotion and copulation (Ahlenius et al., 1987; Damsma et al., 1992), and increased activity in response to salient and arousing stimuli, and one hypothesis for the function of the SNpc is that it may mediate motor responses to rewarding or arousing stimuli (Horvitz, 2000; reviewed in Schultz et al., 1997; Schultz, 1997). Thus, the SNpc may shape learning and performance of sexual behaviors in a context-specific manner. To a

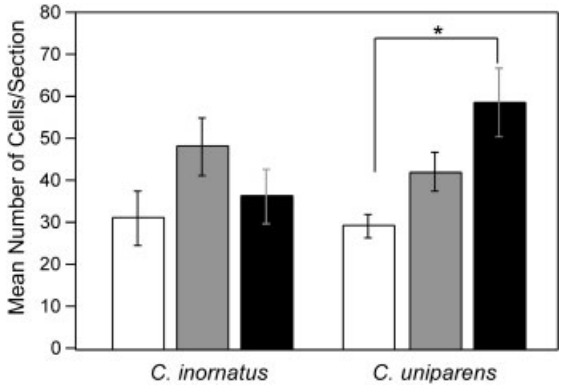

Figure 4 Both species and reproductive state affected the number of cells per section in the SNpc. In *C. uniparens*, the number of TH-ir cells increased across the reproductive cycle, from previtellogenic (white bars) to vitellogenic (gray bars) to postovulatory (black bars), and postovulatory *C. uniparens* individuals had significantly more cells than previtellogenic *C. uniparens* individuals. There was no significant effect of reproductive state on the number of TH-ir cells in *C. inornatus* females. * indicates a significant difference at $p < 0.008$.

postovulatory *C. uniparens* individual, a sexually receptive female may represent a salient or arousing stimulus and the increase in dopamine production in postovulatory *C. uniparens* females in the SNpc may provide either an active or permissive effect on mounting behavior.

Individuals of both species were group-housed prior to the study and therefore had the opportunity to display social and reproductive behaviors as well as other motor behaviors. Previous studies on whiptails indicate that social housing condition can facilitate reproduction (Crews et al., 1986), affect reproductive physiology (Lindzey and Crews, 1988b), and increase the metabolic activity of nuclei involved in the display of male-like sexual behavior (Sakata et al., 2002). Interestingly, the SNpc was one of the few nuclei where TH-ir cell number was modulated by both social housing and intrinsic sexual vigor in *C. inornatus* males (S.C. Woolley, J.T. Sakata, D. Crews, unpublished observations). Because we did not monitor behavior, but rather chose individuals based on reproductive state, we cannot determine whether differences in TH-ir cell number were causally related to species differences in behavior. However, it is possible that the differences in TH-ir cell number are related to specific differences in social or sexual behavior. For example, higher levels of TH in postovulatory *C. uniparens* may reflect or result from the greater propensity of the parthenogen to display male-like behaviors. It will be interesting to determine whether this is the case, and moreover which aspects of social housing may be involved in affecting species differences in TH expression.

The species differences in TH-ir cell number in the SNpc may also be due to differences in the responsiveness of TH-ir cells to steroid hormones. Studies of the relationship between the estrous cycle and dopaminergic function in the striatum of rats have found changes in dopamine synthesis and release depending on reproductive state (reviewed in Becker, 1999). Similarly, work on zebra finches has found that androgens affect dopamine levels in a number of nuclei, including a basal-ganglia-like nucleus, Area X, that is specifically involved in song learning and plasticity (Barclay and Harding, 1990). In whiptails, there are species differences in estrogen regulation of ER and PR in limbic nuclei. For example, estrogen increases PR expression in the periventricular POA in *C. uniparens* but not in *C. inornatus* females (Godwin and Crews, 2002). It would be interesting to determine whether there are species differences in steroid hormone receptor regulation in the SNpc or striatum, and moreover whether these changes may affect TH expression in these areas.

Cnemidophorus inornatus females had significantly more TH-ir cells in the AH, and a similar trend was apparent in the POA. We hypothesized, based on work in amphibians (e.g., Roth et al., 1994), that if ploidy was one of the main factors affecting cell number and size, *C. inornatus* females would have more but smaller TH-ir cells than *C. uniparens* individuals, which is the pattern seen in the AH. However, it is also possible that the difference in cell number is attributable to other behavioral, physiological, or genetic differences between the species. Further investigation of other polyploid species within the *Cnemidophorus* genus would help to resolve whether ploidy was indeed a factor affecting TH-ir cell number in these nuclei.

While there was not a species difference in the number of TH-ir cells in the DH, somal area was larger in *C. uniparens* individuals than in *C. inornatus* females. Thus, parthenogenetic individuals may produce greater quantities of TH in the DH than females of the sexual species. We have found recently that the number of TH-ir cells in the DH is higher in sexually active *C. inornatus* males than in males that are less sexually active (S.C. Woolley, J.T. Sakata, and D. Crews, unpublished observations). These data imply that the level of TH production in the DH may be correlated with an individual's propensity to display male-like sexual behaviors, which is also consistent with the higher levels of TH seen in the DH of the parthenogen relative to females of the sexual species.

The effects of differences in the number or size of TH-ir cells on dopamine synthesis and release or other aspects of neural organization in lizards is unknown. One possibility is that greater numbers of TH-ir cells are associated with greater levels of TH expression and with greater levels of catecholamine synthesis. In mice, for example, it has been demonstrated that individuals from different strains that differ in the number of TH-ir cells in the SNpc also differ in the level of dopamine synthesis and turnover in the striatum (Baker et al., 1980, 1982, 1983; Sved et al., 1984). If indeed there are differences in dopamine synthesis or release in whiptails, there may consequently be effects on the regulation of postsynaptic receptor expression. Investigation of whether the differences in TH expression seen with differences in species or ovulatory state are also correlated with differences in other aspects of catecholaminergic tone would lend additional insight into the role of catecholamines in the display of social behaviors in reptiles. In addition to effects on catecholaminergic tone, species differences in cell size and number may affect other aspects of neural organization. Genome size is positively correlated with cell size across taxa,

and somal area is an important determinant of connectivity and neural morphology (Rakic, 1975; Sultan et al., 2003; Szaro and Tompkins, 1987; Tompkins et al., 1984). The effects of changes in cellular or synaptic organization on behavior have been studied in a number of systems, including the mammalian hippocampus (Meng et al., 2002; Sandstrom and Williams, 2001; Woolley and McEwen, 1992, 1993) and vocal control nuclei of songbirds (DeVoogd et al., 1985; Hill and DeVoogd, 1991; Nottebohm, 1981; Smith et al., 1997). Whether species differences in the size and number of TH-ir cells in whiptails are correlated with other differences in neural structure or organization has yet to be investigated; however, species differences in TH-ir cell size and number have the potential to contribute to behavioral differences between the species and can even serve as a substrate for the evolution of novel neural or behavioral phenotypes.

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