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Tyrosine Hydroxylase Expression Is Affected by Sexual Vigor and Social Environment in Male *Cnemidophorus inornatus*

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ABSTRACT

Although the distribution of catecholamine-synthesizing cells has been described for a variety of taxa, less is known about the functional significance of particular populations in nonmammalian species, especially reptiles. To understand the role of these populations in the display of social behaviors in lizards, we studied the interactive effects of sexual vigor (sexually vigorous vs. sluggish) and social condition (housing in isolation vs. with females) on the number and somal areas of cells expressing tyrosine hydroxylase (TH), a rate-limiting enzyme in catecholamine synthesis, in male whiptail lizards, *Cnemidophorus inornatus*. We found that, regardless of social condition, sexually vigorous males had more TH-immunoreactive (TH-ir) cells in the dorsal hypothalamus (DH) relative to sluggish males. Sexually vigorous males also had more TH-ir cells in the substantia nigra pars compacta (SNpc), but this difference was significant only among males housed with females. Sexually vigorous males that had been housed with females had smaller TH-ir cells in the preoptic area (POA) than vigorous males housed in isolation. On the other hand, no significant differences were found in the anterior hypothalamus. These results highlight the regional heterogeneity in the plasticity of TH expression and suggest that, just as in other species, the DH, SNpc, and POA might be involved in the expression of social behaviors and in behavioral plasticity following social experiences in lizards. J. Comp. Neurol. 476: 429–439, 2004.

Indexing terms: dopamine; substantia nigra; dorsal hypothalamus; preoptic area; sexual behavior; social experience

Catecholamines have been implicated in the display of sexual and aggressive behaviors in many vertebrate species. For example, dopaminergic agonists and antagonists facilitate and inhibit, respectively, the display of courtship and copulatory behaviors in rats (Bitran and Hull, 1987), Japanese quail (Absil et al., 1994; Balthazart et al., 1997), and lizards (Woolley et al., 2001), and dopamine release into the medial preoptic area (mPOA) is critical for the display of copulatory behavior in male rats (Hull et al., 1995). In addition, across taxa, catecholamine-synthesizing cells are found in brain areas critical for the expression of social behavior, including the POA, anterior hypothalamus (AH), and ventral tegmental area (VTA; Smeets and Gonzalez, 2000), and catecholamine synthesis is affected by gonadal steroid hormones in these areas. In birds, frogs, and rodents, the expression of tyrosine hydroxylase (TH), a rate-limiting enzyme in catecholamine synthesis, in nuclei within the POA, the hypothalamus, and the tuberoinfundibular and tuberhypophoseal systems and catecholamine content and turnover within the mPOA and hypothalamus are affected by androgen deprivation and replacement (Gunnet et al., 1986; Barclay and Harding, 1988, 1990; Mitchell and Stewart, 1989; Simerly,

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1989; Sanghera et al., 1991; Chu and Wilczynski, 2002; Wilczynski et al., 2003). Furthermore, adrenal steroid hormones influence TH expression in tuberoinfundibular neurons as well as in neurons of the VTA in rats (Van Loon et al., 1977; Ortiz et al., 1995). Consequently, the production of catecholamines influences the display of social behaviors and interacts with the endocrine system.

Across vertebrate taxa, there appears to be substantial homology in the general distribution of catecholamine-synthesizing cells among vertebrates (for review see Smeets and Gonzalez, 2000). However, there are species differences in the localization of particular populations of catecholamine-synthesizing cells. For example, although there are TH-immunoreactive (TH-ir) cells throughout the telencephalon and diencephalon in reptiles, there is interspecies variability in the specific localization of those TH-ir cells within the POA and hypothalamic nuclei (see, e.g., Smeets, 1994). Moreover, whether there is similarity in the function of specific TH-ir cell populations remains unknown.

Cnemidophorus lizards (Teiidae) offer a useful model system in which to study the role of catecholaminergic cell populations in the expression of social behaviors, because dopamine agonists can induce mounting behavior in gonadectomized individuals (Woolley et al., 2001). Furthermore, interactions with females alter the endocrine, behavioral, and neural metabolic phenotypes in *Cnemidophorus inornatus* males (Lindzey and Crews, 1988b; Sakata et al., 2002). For example, males housed with females have lower androgen and higher corticosterone concentrations than males housed in isolation (Lindzey and Crews, 1988b). Because gonadal and adrenal steroid hormones affect TH expression in mammals (Sanghera et al., 1991; Watanabe et al., 1995; Filipenko et al., 2001) and amphibians (Chu and Wilczynski, 2002), it is possible that heterosexual housing can alter the dopaminergic system in whiptail lizards.

To assess the role of TH-ir cell populations in the display of social behaviors in lizards, we analyzed differences in the size and number of TH-ir cells in the POA, AH, dorsal hypothalamus (DH), and substantia nigra pars compacta (SNpc) of sexually vigorous and sluggish male whiptail lizards, *C. inornatus*, housed either in isolation or with three or four gonadally intact, cycling females. Furthermore, we provide the first detailed description of the distribution of TH-ir neurons in the brain of a teiid lizard (*C. inornatus*).

MATERIALS AND METHODS

Animals, housing, and behavioral testing

C. inornatus males were collected near Sanderson, Texas, during the summer of 2001 under a license from the state of Texas. Males were taken to the laboratory at the University of Texas at Austin and either housed in isolation (25 × 32 × 32 cm; ISOLATE: n = 23) or housed with three or four gonadally intact, cycling females (75 × 32 × 32 cm; HWF: n = 12). In each cage, there was a water dish and at least one wood block to allow for retreat from the light. During the summer, individuals were housed on a 14:10-hour L:D cycle, with temperatures fluctuating from 33°C during the day to 23°C during the night. In November, all individuals were acclimated to conditions resembling hibernation by decreasing photoperiod and temperatures on a weekly basis. During hibernation, males were kept on a photothermal cycle of 8:16 L:D, with temperatures fluctuating from 12.5°C during the day to 10°C during the evening. After 10 weeks in hibernation, photoperiod and daily temperatures were gradually increased on a weekly basis until reaching the summer photothermal regime.

Three additional males were housed with females beginning immediately before emergence from hibernation, and the neural phenotype of these males was not significantly different from that of those housed with females from the summer. Therefore, we pooled males housed with females during emergence from hibernation together with males housed with females since the summer in our statistical analyses (now, n = 15 for HWF males). The results are not altered by the inclusion or exclusion of these males.

Beginning 2 weeks after the onset of the summer schedule, ISOLATE and HWF males were given five daily tests with a receptive female using the protocol described by Sakata et al. (2002). Because group cages were substantially larger than the cages in which ISOLATE males resided, for each screening test HWF males were taken from their group cage and placed into a cage of the same dimensions as the cages of ISOLATE males. To minimize the effects of handling stress on behavior, testing did not commence for at least 2 hours after the transfer of HWF males. To minimize the effect of novelty on courtship behavior (see, e.g., Crews, 1974), HWF males were also placed in these cages for several hours on 2 consecutive days before the first day of screening tests to habituate the males to the cage. ISOLATE males were tested in their home cage.

At least 10 minutes before each test, wood blocks and water dishes were removed from the cage. Thereafter, a receptive female was introduced into the cage, and males were watched for 3 minutes. Females were first screened for receptivity with a sexually vigorous male. In this species, courting males first approach the female, then mount, and then proceed to grip the neck of the female with their jaws while rapidly undulating their pelvis laterally on top of the female. After 1–3 minutes of riding the female, males will intromit (Lindzey and Crews, 1986). If the male failed to mount, tests were terminated at 3 minutes, but, if the male mounted, tests were stopped before the male intromitted. In our laboratory, we have consistently used 3-minute tests to screen for sexual activity under a variety of hormonal states (see, e.g., Lindzey and Crews, 1986, 1988a; Sakata et al., 2002), and, in most cases, sexually vigorous males will mount females within 1 minute of the female's introduction (J.T. Sakata and D. Crews, unpublished data). Males that courted and mounted females on at three of five tests were considered sexually vigorous (n = 11 for ISOLATE males and n = 8 for HWF males), and those that courted and mounted on two or fewer tests were categorized as sexually sluggish (n = 12 for ISOLATE males and n = 7 for HWF males).

Three weeks after the screening tests, all males were killed by rapid decapitation after mild anesthesia (~1 minute in ice). During the 3 weeks before sacrifice, all males were housed in their home cages (HWF males were housed with females, and ISOLATE males remained isolated). We waited for 3 weeks to sacrifice the males to minimize the immediate effects of testing (interactions with females and handling by experimenters) on neural

phenotype. Brains were removed and placed in 4% paraformaldehyde in phosphate-buffered saline for 48 hours at 4°C, then transferred to a 20% sucrose solution overnight. Thereafter, brains were frozen in isopentane and kept at −80°C until processing. The experimental protocol adhered to institutional guidelines and the NIH *Guidelines for the Use of Animals in Research*.

TH immunohistochemistry

Serial 60-μm sections were cut on a cryostat, and two sets of tissue were collected and stored in antifreeze at −20°C. One set was used for TH immunohistochemistry performed on free-floating sections described in this study and sections from all animals in the study were processed simultaneously. All sections from an individual were placed into one well on a 12-well plate. We used four plates, allowing us to run all 38 brains using solutions made from the same stock at the same time. 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 30 minutes at 4°C. After being blocked for 1 hour in 4% normal goat serum, sections were incubated for 72 hours at 4°C with a monoclonal primary antibody (1:600, mouse anti-TH; Chemicon International, Temecula, CA) in 4% goat serum. The antibody has been used in other lizards (Lopez et al., 1992). Sections were then incubated for 2 hours in a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:350; Vector, Burlingame, CA) at room temperature. Immunoreactivity was visualized with 3,3′-diaminobenzedine (DAB; Vector; incubated for 7 minutes). 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. There was no staining on the negative control slides.

Cell counting and analysis

Slides were randomized and coded so that we were blind to social condition and behavioral profile. We counted the number of TH-ir cells in the POA (medial and periventricular), AH, DH, and SNpc. Nuclei were delineated according to Young et al. (1994). Cells were counted within a nucleus by using a ×40 objective on a Nikon Eclipse E800 microscope. For each individual, we counted all cells bilaterally in each nucleus on all sections in which the nucleus was present. Two to four sections were counted per nucleus per individual, and the number of cells was averaged for each side of the brain 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 with a nucleator program (MicroBrightField, Colchester, VT). Sections were imaged with a Zeiss microscope fitted with a Ludl Electronic Products (LEP) MAC 2002 motorized stage, an Optronics DEI 750 camera, and a Dell Pentium III XPS B733r computer. The nucleator program required the user to identify a point near the center of the cell. From that point, eight rays were extended, and the intersection of each ray with the boundaries of the cell was marked by the user, and the somal area was calculated based on the cell boundaries.

Photomicrographs were taken with a Nikon Eclipse E800 microscope equipped with a Spot 1400 slider camera (Diagnostic Instruments, Sterling Heights, MI) and a Power Macintosh G3 with Spot imaging software version 3.5.9 (Diagnostic Instruments). Contrast and brightness were adjusted in Adobe Photoshop to enhance the visibility of TH-ir fibers.

Statistical analysis

All data on TH-ir cell number and size were normally distributed. Therefore, for each parameter in each nucleus, we analyzed the data with a two-way analysis of variance (ANOVA), with sexual vigor (vigorous vs. sluggish) and social condition (ISOLATE vs. HWF) as the independent variables and set $\alpha = 0.05$. If there was a significant interaction between sexual vigor and social condition, we performed four planned contrasts using Studentized *t*-tests: 1) vigorous HWF vs. sluggish HWF, 2) vigorous ISOLATE vs. sluggish ISOLATE, 3) vigorous HWF vs. vigorous ISOLATE, and 4) sluggish HWF vs. sluggish ISOLATE. We adjusted our α to 0.0125 (Bonferonni correction) for all pairwise contrasts to control for the multiple pairwise comparisons. Statistics were analyzed in JMP 3.2 (SAS Institute) for the Macintosh.

RESULTS

Description of TH-ir cell bodies and fibers in the whiptail brain

The mean number of TH-ir cells counted per section was highest in the SNpc (mean ± SE 48.3 ± 3.1) and AH (51.8 ± 1.9) and lower in the DH (27.1 ± 1.6) and POA (17.7 ± 1.2). Somal areas of TH-ir cells were largest in the SNpc (mean ± SE 145.9 ± 3.1 μm^2), moderate in the DH (113.1 ± 3.0 μm^2) and AH (105.9 ± 1.5 μm^2), and smallest in the POA (93.2 ± 1.6 μm^2). We found that the TH-ir cells in the AH and POA were similar in size to cells in those nuclei described by Wade and Crews (1992).

There was a dense accumulation of TH-ir fibers and varicosities in the striatum and nucleus accumbens, which we presume were fibers of SNpc and VTA neurons (Fig. 1A; Gonzalez et al., 1991), as well as in the dorsal ventricular ridge, lateral cortex, and anterior septum. The most rostral population of TH-ir cell bodies was in the POA, where TH-ir cells were found medially near the third ventricle in the periventricular POA (PvPOA; Figs. 1B, 2A) as well as lateral to the PvPOA, within the mPOA, and in more ventral positions within the suprachiasmatic nucleus (SC). Cells in the PvPOA were oriented along the dorsoventral axis and sent TH-ir fibers ventrally toward the SC, medially toward the third ventricle, or dorsally.

A population of TH-ir cells emerged just caudal to the mPOA in the preoptic recess (POr) and was located ventrally in more rostral sections and spread dorsally, though it remained medial in more caudal sections. A catecholamine population in the POr seems to be absent in *Podarcis s. sicula*, *Varanus exanthematicus* (Smeets, 1988), and *Gecko gecko* (Smeets et al., 1986) but is present in *Anolis carolinensis* (Lopez et al., 1992), the Caiman crocodile *Caiman crocodilis* (Brauth, 1988), and the red-eared slider turtle *Trachemys scripta elegans* (Smeets et al.,

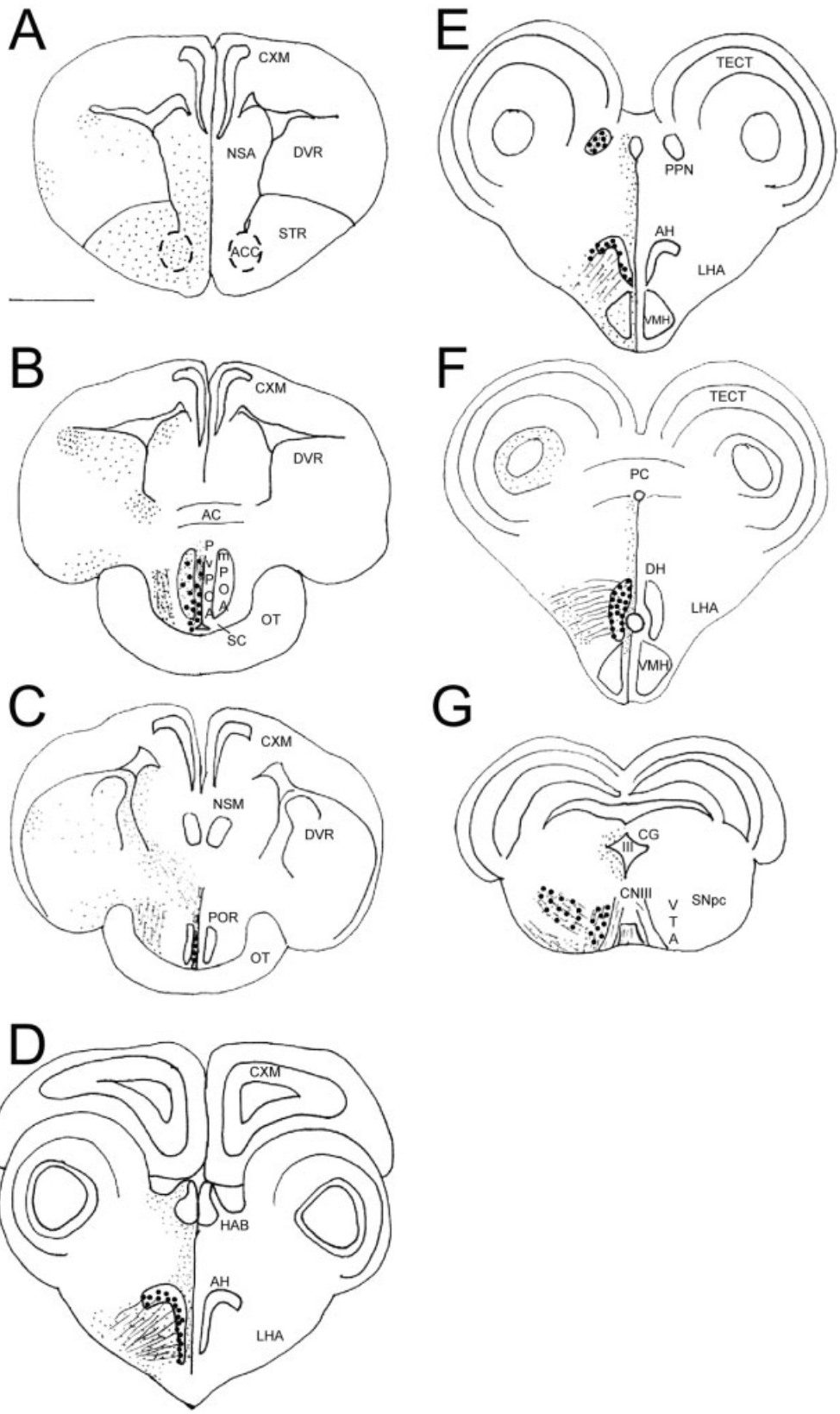

Fig. 1. **A–G:** Camera lucida drawings of the locations of TH-ir cells and fibers in the forebrain, diencephalon, and mesencephalon of male whiptail lizards (*Cnemidophorus inornatus*). Solid circles indicate the presence of TH-ir cell bodies, whereas stippled areas and fine lines indicate the presence of TH-ir varicosities and fibers. AC, anterior commissure; ACC, nucleus accumbens; AH, anterior hypothalamus; CG, central gray; CNIII, cranial nerve three; CXM, medial cortex; DH, dorsal hypothalamus; DVR, dorsal ventricular ridge; HAB, habenula; LHA, lateral hypothalamic area; mPOA, medial preoptic area; NSA, anterior nucleus septum; NSM, medial septum; OT, optic tract; PC, posterior commissure; POr, preoptic recess; PPN, posterodorsal pretectal nucleus; PvPOA, periventricular preoptic area; SC, suprachiasmatic nucleus; SNpc, substantia nigra pars compacta; STR, striatum; TECT, tectum; VMH, ventromedial hypothalamus; VTA, ventral tegmental area; III, third ventricle. Scale bar = 1 mm.

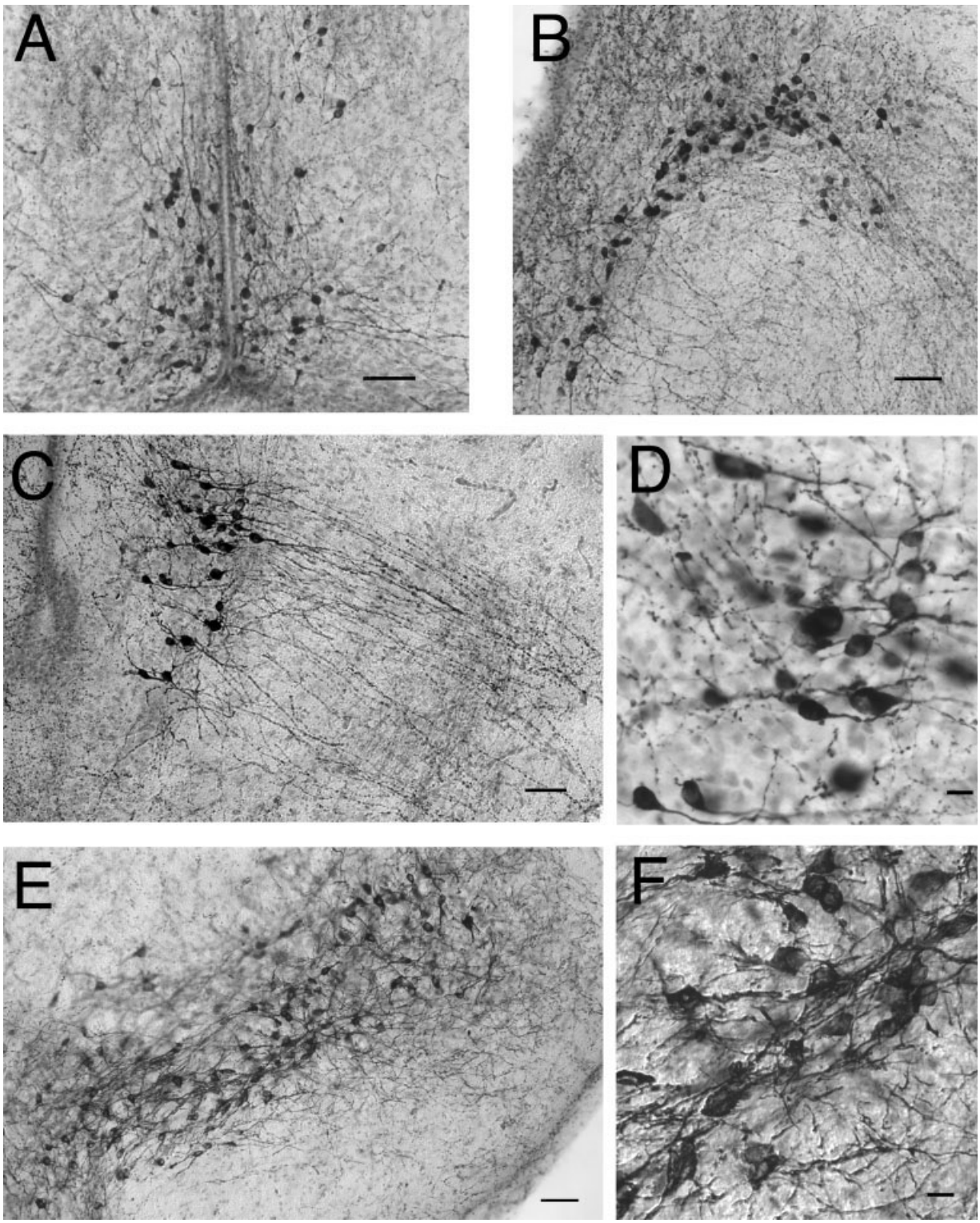

Fig. 2. Photomicrographs of TH-ir staining in the POA (**A**), AH (**B**), DH (**C,D**), and VTA (medially located cells in E) and SNpc (**E,F**) of *Cnemidophorus inornatus* males. Images were taken of sections with reddish-brown DAB-stained TH-ir cells and a purple-blue cresyl violet counterstain. To enhance to appearance of the TH-ir cells, a blue filter was implemented using Spot imaging software version 3.5.9 (Diagnostic Instruments) during image acquisition, and images were converted to gray scale. Consequently, DAB-stained cells and fibers appear black, whereas Nissl-stained cells appear gray or, in many cases, are filtered out entirely. Scale bars = 50 μm in A–C,E; 10 μm in D,F.

1987; Smeets, 1988; Smeets and Steinbush, 1990). One possibility is that the POr population was lost in a common ancestor to the Gekkonidae, Teiidae, Lacertidae, and Varanidae families. Thus, the presence of the POr population in the teiid *C. inornatus* may be a newly derived characteristic.

The AH in *Cnemidophorus*, as designated by Young et al. (1994), appears similar to the anterior hypothalamic-periventricular hypothalamic-lateral hypothalamic area complex (AH:PH:LHA) in *A. carolinensis* (Lopez et al., 1992) as well as the periventricular hypothalamus in *G. gecko* (Smeets et al., 1986; Figs. 1D, 2B). The TH-ir cells in the AH spread from ventral to dorsal and medial to lateral in a line that followed the ventrolateral edge of the anterior hypothalamic nucleus (Fig. 1D). Cells in the ventromedial portion of the AH were slightly larger than those in the dorsolateral portion. TH-ir cells in the AH population sent dense projections ventrolaterally into the lateral hypothalamic area.

The DH population was located just caudally and dorsally to the AH population at the level of the periventricular organ (PVO; Figs. 1E, 2C,D). In the rostral portion of the DH, cells were aggregated together dorsolaterally to the PVO, whereas, in more caudal sections, additional cells extended ventrally and lined the medial and lateral edge of the nucleus. The cells in the DH sent TH-ir fibers both medially toward the PVO and laterally toward the lateral hypothalamus.

As has been documented in other reptiles, there were TH-ir cells in the pretectal posterodorsal nucleus (PPN; Fig. 1F) that were smaller and stained less intensely than cells in the rest of the brain. In the midbrain, TH-ir cells were visible near the third cranial nerve, in what is presumed to be the reptilian VTA, and lateral to the VTA cells was the SNpc population. In the SNpc, both cells and projections were oriented ventromedially to dorsolaterally (Fig. 2E,F). TH-ir fibers were visible within the SNpc, between the SNpc and VTA, and laterally to the SNpc (Fig. 1G). The SNpc was the most caudal nucleus that we investigated.

TH-ir cell counts

In the DH, sexually vigorous males had significantly more TH-ir cells than sluggish males regardless of social condition [$F(1,30) = 10.5.0$, $P = 0.003$; Fig. 3A]. In the SNpc, the interaction between sexual vigor and social condition approached significance [$F(1,21) = 4.23$, $P = 0.052$; Fig. 4A]. Given this trend, we conducted planned contrasts, which revealed that, among HWF males but not ISOLATE males, sexually vigorous males had more TH-ir cells than sluggish males, although this effect was not significant with our adjusted α' ($P = 0.041$). There were no significant effects of sexual vigor or social condition on the number of TH-ir cells in the POA or AH.

To bolster our confidence in the relationship between sexual vigor and TH-ir cell number in the DH and SNpc, we looked at the correlation between the number of tests in which courtship was displayed (0–5) and the number of TH-ir cells. In the DH, when all males are included, the correlation is positive and highly significant ($r = 0.56$, $n = 34$, $P < 0.001$; Fig. 3B). In the SNpc, correlations were analyzed separately for HWF and ISOLATE males, and we found that the correlation was significant and positive only for HWF males (HWF: $r = 0.687$, $n = 13$, $P = 0.009$; ISOLATE: $r = -0.21$, $n = 12$, $P = 0.516$; Fig. 4B).

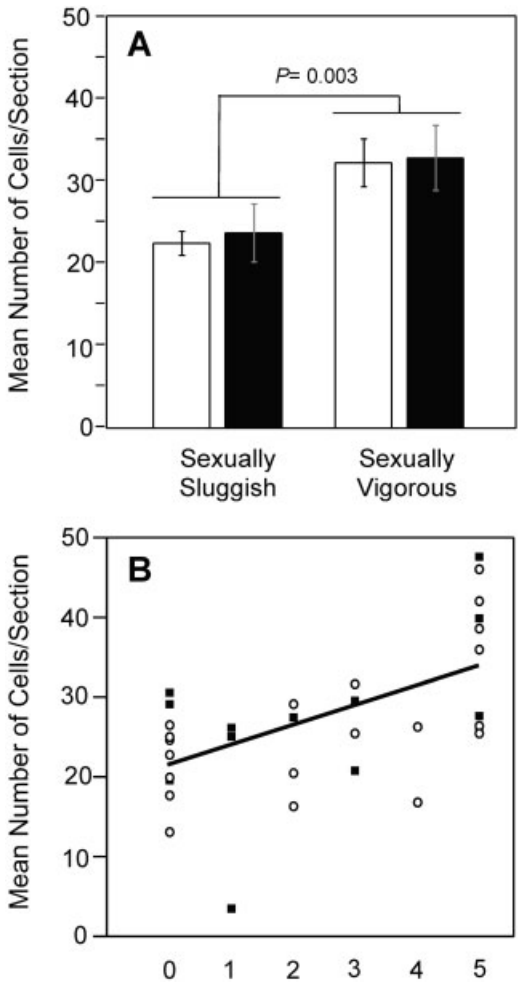

Fig. 3. In the dorsal hypothalamus, regardless of housing condition, sexually vigorous males had more TH-ir cells than sexually sluggish males. **A:** Solid bars are males that were housed with females; open bars are isolated males. Presented are means ± SEM. **B:** Significant positive correlation between sexual vigor and the number of TH-ir cells ($r = 0.56$, $P < 0.001$) for both males housed with females (squares) and males housed in isolation (circles).

TH-ir cell size

In the POA, the somal area of TH-ir cells was significantly larger in ISOLATE males than in HWF males [$F(1,26) = 5.2$, $P = 0.020$; Fig. 5]. Moreover, the interaction between sexual vigor and social condition on somal area in the POA approached significance [$F(1,26) = 4.2$, $P = 0.051$], and planned contrasts revealed that sexually vigorous HWF males had smaller somal areas relative to vigorous ISOLATE males ($P = 0.006$), but this difference was not significant among sexually sluggish males (Fig. 5). In addition, there was a trend for smaller TH-ir somal areas in sexually vigorous HWF males than sluggish HWF males, although this difference did not reach statistical significance with the adjusted α' ($P = 0.032$). There were no significant differences among ISOLATE males. In the SNpc, the interaction between sexual vigor and social condition also approached significance [$F(1,21) = 3.8$, $P = 0.063$], and, although no contrasts were significant with

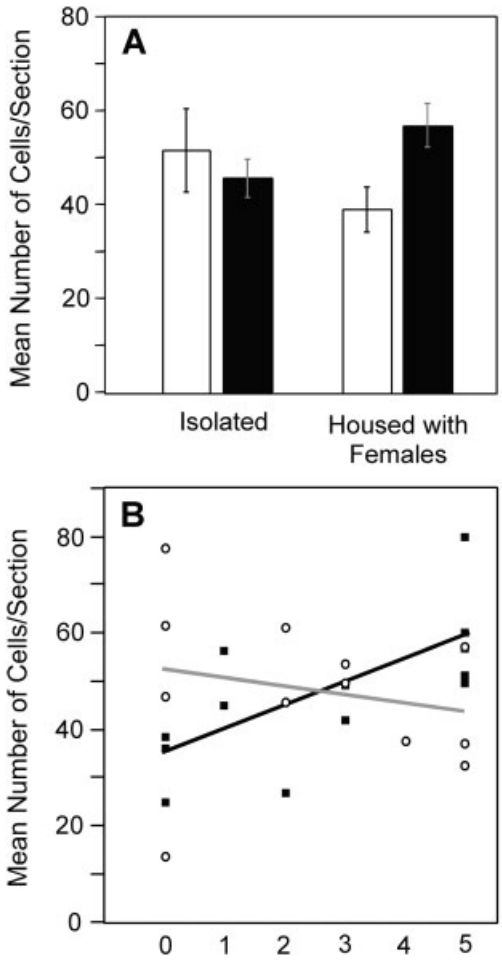

Fig. 4. In the substantia nigra pars compacta, there was an interaction between sexual vigor and social condition affecting the number of TH-ir cells in male whiptail lizards, *Cnemidophorus inornatus*. **A:** There was a trend toward more TH-ir cells in sexually vigorous males (solid bars) than sluggish males (open bars) when they were housed with females but not when they were housed in isolation. Presented are means ± SEM. **B:** There was a significant positive correlation between sexual vigor and TH-ir cell number among males housed with females (squares, black line; $r = 0.687$, $P = 0.009$) but not isolated males (circles, gray line; $r = -0.21$, $P = 0.516$).

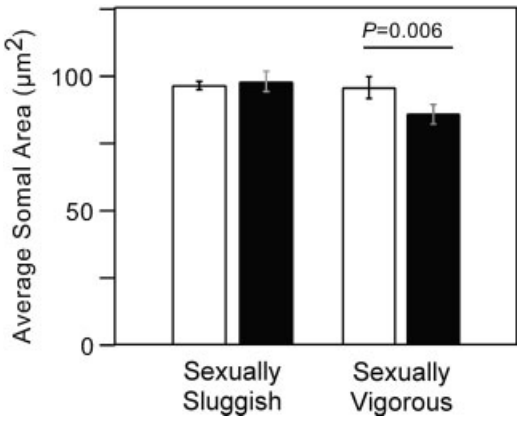

Fig. 5. There was an interaction between sexual vigor and social condition on TH-ir somal areas in the preoptic area of male whiptail lizards, *Cnemidophorus inornatus*. Among sexually vigorous males, males that were housed with females (solid bars) had smaller somal areas than isolated males (open bars). Presented are means ± SEM.

the adjusted α', there was a nonsignificant trend for larger somal areas in the SNpc of sexually vigorous HWF males than sluggish HWF males ($P = 0.023$). There was no significant effect of sexual vigor or social condition on TH-ir cell soma size in the AH or DH.

Just as with cell counts, we correlated the number of tests with courtship (0–5) and mean somal area of TH-ir cells in the POA and SNpc, and, in both cases, HWF and ISOLATE males were analyzed separately. In both nuclei, correlations were not significant for either HWF or ISOLATE males, which is consistent with our means analysis.

DISCUSSION

Catecholamines modulate the expression of social and courtship behaviors in a variety of vertebrates, including whiptail lizards (Bitran and Hull, 1987; Hull et al., 1995, 1997; Balthazart et al., 1997; Woolley et al., 2001), and catecholamine production and release in areas such as the mPOA, nucleus accumbens, and paraventricular nucleus of the hypothalamus are critical for the display of sexual behavior in rats (Damsma et al., 1992; Hull et al., 1995; Melis et al., 2003). Therefore, intrinsic differences in the expression of TH, the rate-limiting enzyme in catecholamine synthesis, could have profound implications for the display of social behavior.

Here we analyzed differences in the number and somal area of TH-ir cells between sexually vigorous and sluggish male whiptail lizards, *C. inornatus*, which were either housed in isolation (ISOLATE males) or housed with females (HWF males). The number of TH-ir cells in the DH was greater in sexually vigorous males than in sexually sluggish males, regardless of housing condition, suggesting a role in the regulation of courtship behavior (Fig. 3A,B). In the SNpc, there was a correlation between sexual vigor and the number of TH-ir cells among HWF males but not ISOLATE males (Fig. 4A,B). In the POA, somal areas of TH-ir cells were smaller in sexually vigorous HWF males relative to sexually vigorous ISOLATE males (Fig. 5). No differences were observed in the AH. Therefore, there was regional heterogeneity in the degree to which sexual vigor and social housing influenced TH expression, which could be related to regional heterogeneity in factors such as sex steroid hormone receptor expression or cAMP response element-binding protein (CREB; Goodman, 1990; Lazaroff et al., 1995).

Our most robust finding was that the number of TH-ir cells in the DH was significantly positively related to sexual vigor, regardless of housing condition (Fig. 3A,B). There are relatively few studies that document neural differences between sexually vigorous and sluggish males (see, e.g., Harding and Feder, 1976; Clark et al., 1985; Alexander et al., 1993; Mendonca et al., 1996; Prince et al., 1998), and this difference in the DH suggests a neurochemical modulation of individual differences in behavior. This is intriguing because the DH has not previously been implicated in the expression of courtship behavior in reptiles, and in this species the DH does not express high

levels of androgen receptor (AR) mRNA (Young et al., 1994). In Japanese quail, copulation increases FOS expression in the PVO and periventricular hypothalamus, two nuclei that may be similar to the DH in lizards (Meddle et al., 1999). Similarly, mating increases neural activity in the dorsomedial hypothalamus in primates, which is togopraphically similar to the DH in *C. inornatus*, and, like the DH of *C. inornatus* (Young et al., 1994), expresses estrogen receptor (ER) and progesterone (PR) mRNA but not AR mRNA (Simerly et al., 1990; Hagihara et al., 1992; Shughrue et al., 1997). In amphibians, the TH-ir cell population near the PVO may be homologous to the mammalian zona incerta, or A13 population (Milan and Puelles, 2000; Sanchez-Camacho et al., 2001a,b, 2002), which has been implicated in the display of masculine behaviors in mammals (Perachio et al., 1979; Maillard and Edwards, 1991; Maillard et al., 1994; Heeb and Yahr, 1996). Given our current results, it will become increasingly important to study the development, chemoarchitecture, and connectivity of the DH to identify homologous nuclei in other species and target nuclei for the effects of catecholamines.

In the SNpc, the number of TH-ir cells was positively correlated with the level of sexual vigor of HWF males but not ISOLATE males (Fig. 4A,B). This finding is analogous to the correlation between reproductive state and TH-ir cell number in the SNpc of group-housed parthenogenetic whiptail lizards, *C. uniparens* (Woolley and Crews, 2004). After ovulation (when individuals have high progesterone and low estradiol concentrations), parthenogenetic whiptails are more likely to display male-typical courtship behavior (for review see Woolley et al., 2004), and postovulatory parthenogens have more TH-ir cells in the SNpc relative to parthenogens without follicles. Together, these two results suggest a modulatory role of the SNpc on the expression of male-typical courtship behavior in *Cnemidophorus* lizards.

Although the SNpc is traditionally thought of as an area primarily controlling motor output, there is evidence that the SNpc and its dopaminergic efferents to the striatum are also involved in the expression of sexual behavior as well as stimulus-reward associations. In rats, dopamine synthesis and release in the striatum increase more with copulation than with locomotion alone (Ahlenius et al., 1987; Damsma et al., 1992), and the activity of neurons in the SNpc increases both with the presentation of rewards as well as in response to stimuli that predict rewards (Schultz et al., 1993, 1997). Sexually vigorous *C. inornatus* males initiate copulation sooner and are more likely to copulate with a sexually receptive female than sluggish males, and vigorous HWF males continue to copulate longer after castration than vigorous ISOLATE males (Sakata et al., 2002). It is possible that sexual experience with females increases the expression of TH in the SNpc and that this increase enhances the reward value of copulatory experiences with females. Furthermore, this heightened reinforcement value of females could underlie the increased capacity to court females in the absence of androgens.

Mean somal areas of TH-ir cells in the POA were also affected by an interaction between sexual vigor and social condition: Sexually vigorous HWF males had smaller TH-ir cells than vigorous ISOLATE males (Fig. 5). The function of TH-ir cells in the POA is not well understood. Interestingly, a population of TH-ir cells in the POA is absent in a number of other lizards that have been studied, including *G. gecko* (Smeets et al., 1986), *P. s. sicula*, and *V. exanthematicus* (Smeets, 1988), whereas there is a prominent population of TH-ir cell bodies in the POA of the iguanid lizard *A. carolinensis* (Lopez et al., 1992). Species comparisons investigating similarities and differences in behavioral and neural phenotypes might lend insight into the function of this popoulation of TH-ir cells in lizards.

Because housing with females enhances the difference in TH-ir cell number in the SNpc between sexually vigorous and sluggish males, and because housing affects somal areas of TH-ir cells in the POA among vigorous males, we propose that these differences might be driven by sociosexual interactions with females. Males housed with females had the opportunity to mount, intromit, and ejaculate, whereas ISOLATE males were allowed to mount females only during screening tests. Thus, the difference in TH expression in the POA between high courting HWF and ISOLATE males might result from differences in whether males were able to intromit and ejaculate. Similarly, differences in TH expression among HWF males in the SNpc might also be related to differences in behavior: Although sluggish HWF males had ample opportunity to copulate with females, given their lower level of sexual vigor, they may have copulated less frequently than sexually vigorous HWF males. Intromission and ejaculation experiences induce greater changes in motivational and sexual behaviors than mounting alone (Sheffield et al., 1951; Ware, 1968; Whalen, 1968; Lopez et al., 1999) and may have greater effects on neural phenotype in the SNpc and POA. Information on interactions between HWF males and females in the home cage would be useful, but, given the length of our manipulation, we did not monitor copulatory behavior in the home cage and cannot verify whether males with different levels of sexual vigor interacted with females differently. Moreover, it is also possible that other aspects of the social interactions with females aside from copulatory experiences contributed to neural differences in both areas.

On a related note, we do not know how experience during screening tests contributed to differences in areas such as the DH and SNpc. We attempted to minimize the influence of recent experience by stopping tests after mounting and by waiting for 3 weeks before euthanasia. We cannot rule out the possibility that experience during the screening tests might have affected TH expression in the DH. However, despite the dramatic differences in social experience that result from being housed with females, sexually vigorous HWF and ISOLATE males did not differ in TH expression in the DH, supporting the notion that differences in the DH reflect differences in intrinsic sexual vigor rather than recent experience.

The endocrine milieu affects catecholaminergic synthesis, release, and reception in a number of species. For example, in *Rana pipiens*, androgen manipulations affect the number of TH-ir cells in the POA (Chu and Wilczynski, 2002; Wilczynski et al., 2003). *C. inornatus* HWF males have lower concentrations of androgens than do ISOLATE males (Lindzey and Crews, 1988b), and AR mRNA is robustly expressed in the POA (Young et al, 1994). Thus, it is possible that the smaller somal areas of TH-ir cells in vigorous HWF males are associated with differences in endocrine profile. Similarly, the SNpc of rodents and birds contains sex steroid hormone receptors (Maney et al., 2001; Beyer et al., 2002; Curran-Rauhut

and Peterson, 2002; Creutz and Kritzer, 2002; Ravizza et al., 2002; Ivanova and Beyer, 2003). Although it is unknown whether vigorous and sluggish males have endocrine differences if they are housed with females, such endocrine differences could have contributed to the interactive effects found in the SNpc. The need to study the effects of gonadal steroid hormones on TH-ir expression in both the POA and the SNpc in male whiptails is evident.

We anticipated that we would find differences in TH-ir cell phenotype in the AH between sexually vigorous and sluggish males or between ISOLATE and HWF males, given that neural metabolism in the AH increases during copulation in male whiptail lizards (Rand and Crews, 1994) and that the AH is replete with AR mRNA in whiptail lizards (Young et al., 1994) as well as in other lizard species (Moga et al., 2000; Tang et al., 2001; Rosen et al., 2002). However, the lack of differences among males with different behavioral phenotypes is consistent with the finding that the number and size of TH-ir cells in the AH does not change as a function of reproductive state in *C. inornatus* females or in the parthenogenetic *C. uniparens* despite changes in reproductive behaviors (Woolley and Crews, 2004).

An important interpretation issue arises from our behavioral testing paradigm. Whereas ISOLATE males were tested in their home cages, HWF males were transferred to a cage that was identical to the cages of ISOLATE males for screening. Therefore, variation in behavior during testing could also be due to individual differences in reactivity to handling and cage transfer for HWF males, and neural differences among HWF males might reflect differences in reactivity to novelty or handling stress rather than differences in sexual vigor per se. We tried to minimized this effect by allowing males to habituate to the new cage for 2 days leading up to the screening tests and by starting behavioral tests 2 hours after transfer into the test cage. Despite these precautions, because ISOLATE males were not handled, neural differences between vigorous and sluggish males could represent different phenotypes in ISOLATE males relative to HWF males. Furthermore, even though the relationship between sexual vigor and TH expression in the DH is not dependent on social housing, it remains possible that TH expression in the DH of HWF males is also related to reactivity to stressors.

Finally, it will be important to understand the consequences of differences in the number and size of TH-ir cells on catecholamine synthesis. One possibility is that greater levels of TH expression are associated with greater levels of catecholamine synthesis, and, in some instances, individual differences or genetic or pharmacological manipulations that result in differences in TH cell number also result in lower levels of dopamine synthesis and release in rats and mice (Baker et al., 1980, 1982; Sved et al., 1984; Iwata et al., 2000; Althini et al., 2003). For example, strain differences in mice in the number of TH-ir cells in the SNpc are correlated with differences in the level of dopamine synthesis and turnover in the striatum (Baker et al., 1980, 1982; Sved et al., 1984). Likewise, persistent treatment with the dopamine agonist apomorphine results in decreases in the number of TH-ir cells as well as decreases in striatal dopamine release (Althini et al., 2003) in rats. Given the multiple levels at which TH is regulated (transcriptional, translational, phosphorylation, etc.; for review see Kumer and Vrana, 1996), it will be interesting to understand better what changes in enzyme expression mean for this species. Moreover, it will be important to assess behavioral correlates of variation in the regulation of catecholamine release or expression of postsynaptic receptors in this species.

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