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Heterosexual Housing Increases the Retention of Courtship Behavior Following Castration and Elevates Metabolic Capacity in Limbic Brain Nuclei in Male Whiptail Lizards, *Cnemidophorus inornatus*

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In male vertebrates the display of courtship behavior depends on the presence of testicular androgens. However, social experiences in adulthood can alter the hormonal dependence of courtship behavior in a variety of species, and we have previously proposed that these behavioral changes are linked to changes in neural metabolic capacity (cytochrome oxidase activity). Here we investigated the effects of prior social experience (housing with females vs housing in isolation) on the retention of courtship behavior following gonadectomy and on cytochrome oxidase (CO) activity in male little striped whiptail lizards, *Cnemidophorus inornatus*. In Experiment 1, we found that males that were previously housed with females (HWF males) continued to display courtship behavior longer after castration than males previously housed in isolation (ISOLATE males). This is similar to the behavioral plasticity found in rodents and cats. On the other hand, courtship behavior while gonadally intact was indistinguishable between HWF and ISOLATE males. Because all males were housed individually following castration, the difference is due to different social experiences prior to castration. In Experiment 2, we found that gonadally intact HWF males had significantly elevated CO activity in the preoptic area, amygdala, and anterior and ventromedial hypothalamic areas relative to intact ISOLATE males. No significant differences in metabolism were found in the lateral septum, lateral hypothalamus, and habenula or in hindlimb muscle, suggesting that the increase in metabolism is specific to brain nuclei involved in courtship behavior. Altogether, this demonstrates that elevations in metabolic capacity correlate with experience-dependent increases in robustness to castration.

Key Words: **social experience; castration; cytochrome oxidase; whiptail lizard; courtship behavior.**

A classic problem in behavioral endocrinology deals with individual and species differences in the retention of copulatory behaviors following castration. For example, Beach (1947), Aronson (1959), and Hart (1974) speculated on the neural mechanisms underlying species differences in the capacity to display sexual behavior following castration. Social experiences, both early and late in life, can significantly alter the dependence of courtship behavior on the presence of androgens (Stern, 1990; reviewed in Meisel and Sachs, 1994) and contribute to individual differences in postcastration courtship behavior. For instance, male cats given sexual experience in adulthood continue to copulate longer following castration than do naïve males (Rosenblatt and Aronson, 1958a,b), and similar effects of social experiences in adulthood have been documented in other mammalian species (Manning and Thompson, 1976; Larsson, 1978; Lisk and Heimann, 1980; Retana-Marquez and Velazquez-Moctezuma, 1997; Phelps, Lydon, O'Malley, and Crews, 1998). In other words, in a variety of male mammals, sociosexual experience in adulthood engenders an increased robustness to castration. The degree to which this social plasticity is evolutionarily conserved is unknown, and few have studied this phenotype in nonmammalian vertebrates. Because comparative studies lend insight into the evolutionary history of and selection pressures on the phenotype of interest (Harvey

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and Pagel, 1991) and because lizards offer an important evolutionary model system because of the diversity of reproductive modes and strategies (reviewed Gans and Crews, 1992), the investigation of social plasticity in lizards is an important endeavor.

Whereas a number of studies have characterized the behavioral effects of experience on the retention of courtship behavior following castration, there have been relatively few investigations into the mechanism underlying these experience-dependent changes. We have proposed that experience-dependent changes in neural metabolic capacity in the preoptic area and amygdala are causally linked to these behavioral changes (Sakata, Gupta, and Crews, 2001). To date, we have found a positive correlation between heightened metabolic capacity in the limbic system and heightened robustness to castration in male rats and leopard geckos (Crews, Coomber, and Gonzalez-Lima, 1997; Sakata, Gupta, Chuang, and Crews, 2002a; Sakata, Gonzalez-Lima, Gupta, and Crews, 2002b). Here we present two studies assessing the effect of recent social experience while gonadally intact both on the retention of courtship behavior following castration and on metabolic capacity male little striped whiptail lizards, *Cnemidophorus inornatus*. Metabolic capacity was measured using quantitative histochemistry of the activity of cytochrome oxidase, a rate-limiting enzyme in oxidative phosphorylation (Gonzalez-Lima and Garrosa, 1991). Energy consuming mechanisms that deplete ATP lead to an up-regulation in the synthesis of this enzyme (Wong-Riley, 1989), and therefore, cytochrome oxidase histochemistry is suited for measuring long-lasting changes in brain metabolic capacity due to long-term experimental manipulations (Gonzalez-Lima and Cada, 1998). We found that, relative to males housed in isolation, males housed with females (HWF males) continued to show courtship behavior longer after castration (Experiment 1) and that gonadally intact HWF males had elevated metabolic capacity in limbic nuclei known to modulate male courtship behavior (Experiment 2).

METHODS

Experiment 1: Effects of Housing with Conspecific Females on Courtship Behavior While Intact and Following Castration

We collected *C. inornatus* males near Sanderson, Texas, during the summer of 1998 after obtaining state-issued collecting licenses. Males were taken to the lab at the University of Texas at Austin and either housed in isolation (25 × 32 × 32 cm)(n = 77) or housed with 3 or 4 intact, cycling females (75 × 32 × 32 cm)(n = 13). Only 13 males were housed with females because of the paucity of females collected in the field. 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 L:D light 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 8:16 L:D photothermal cycle, with temperatures fluctuating from 25°C during the day to 12.5°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.

Beginning 2 weeks after the onset of the summer schedule, isolated males (ISOLATE) and HWF males were given daily tests with a receptive female. 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 the same dimensions as those of ISOLATE males. To minimize the effects of handling stress on behavior, testing did not commence for at least 2 h after the transfer of HWF males. HWF males were also put in these cages for several hours on 2 consecutive days before the first day of screening tests to habituate the males to the cage; this served to minimize the effect of novelty on courtship behavior (e.g., Crews, 1974). ISOLATE males were tested in their home cage.

At least 10 min before the test, we removed wood blocks and water dishes from the cage. Thereafter, we introduced a receptive female into the cage and watched the males for 3 min. 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 min of riding the female, males will intromit the female (Lindzey and Crews, 1986). If the male failed to court, we terminated the test at 3 min, but if the male courted, we stopped the test before the male attempted intromission. In our lab we have consistently used 3-min tests to screen for sexual activity under a variety of hormonal states (e.g., Lindzey and Crews, 1988; Crews, Godwin, Hartman, Grammer, Prediger, and Sheppherd, 1996), and in most cases, sexually active males will mount females within 1 min of the

introduction of the female (J. T. Sakata and D. Crews, unpublished data).

During each test we recorded the mount and neck grip latencies. Mount latency was defined as the interval between the introduction of the female and the first mount, whereas neck grip latency was defined as the interval between first mount and first neck grip. Individuals that did not show mounting or neck gripping behavior were not assigned a latency score. We administered five screening tests to ISOLATE males but gave HWF males 14 screening tests to provide them additional sociosexual experience.

After the last screening test, males were castrated under cold anesthesia. We castrated only males categorized as sexually active (i.e., ISOLATE, $n = 35$; HWF, $n = 10$). Though 45 ISOLATE males were categorized as sexually active (see Results), 10 sexually active males were transferred to a different study. Males were considered sexually active if they mounted stimulus females in at least 50% of their screening tests. Following castration HWF males were not returned to their home cage with intact females but were subsequently housed in isolation in the same cages in which they had received their screening tests. ISOLATE males were returned to their home cages. Beginning 3 days after castration, we tested males daily for 20 days with sexually receptive females in a manner identical to that prior to castration.

Experiment 2: Effects of Housing with Conspecific Females on Metabolic Capacity (Cytochrome Oxidase Activity) in the Limbic System

We collected *C. inornatus* males near Sanderson, Texas, during the summer of 2000 after obtaining state-issued collecting licenses. *C. inornatus* males were housed in isolation ($n = 24$) or with 2 or 3 females ($n = 10$). Following a period of hibernation identical to that described in the previous experiment, males were brought into a summer photothermal cycle. Three of the HWF males died during hibernation, so just after emergence from hibernation, some males ($n = 6$) were removed from isolation and housed with females for at least 3 weeks. No differences in behavior were found between males housed with females before hibernation and immediately after the period of torpor. Furthermore, no significant differences were found between these groups of HWF males in any brain area except for the lateral hypothalamus (see Results). Consequently, all HWF males were pooled in the analyses.

Males (HWF, $n = 13$; ISOLATE, $n = 12$) were given five daily screening tests with receptive females approximately 1 month following the switch to the summer photothermal cycle. This allowed time for males recently housed with females to acquire social experience. As found in Experiment 1 (see Results), there were no significant differences in the proportion of males categorized as sexually active between HWF and ISOLATE males ($\chi^2_1 = 0.0$, $P = 0.870$). After the last screening tests, HWF and ISOLATE males were sacrificed by rapid decapitation. Brains were quickly removed and frozen in isopentane. Muscle from the left hindlimb was also rapidly collected and frozen in isopentane. Tissues were kept at −80°C and sectioned at 20 μm. Slides were stored at −40°C until processing for cytochrome oxidase (CO) activity.

Detailed protocols for CO histochemistry have previously been published (Gonzalez-Lima and Garrosa, 1991). Briefly, slides were first treated in 10% sucrose phosphate buffer (0.1 M, pH 7.6) containing 0.5% glutaraldehyde for 5 min. This step facilitates the adherence of sections to slides and does not affect the enzymatic activity of CO, as demonstrated empirically in Gonzalez-Lima and Cada (1998). Slides were then rinsed 3× in 10% sucrose phosphate buffer (5 min each) and then incubated for 10 min in Tris buffer (0.05 M, pH 7.6) containing 275 mg/liter of cobalt chloride, 10% sucrose, and 0.5% dimethylsulfoxide (DMSO). Slides were subsequently rinsed for 5 min in phosphate buffer and then incubated at 37°C for 60 min in an oxygen-saturated reaction solution containing 350 mg of diaminobenzidine tetrahydrochloride, 52.5 mg of cytochrome c, 35 g of sucrose, 14 mg of catalase, and 1.75 ml of DMSO in 700 ml of phosphate buffer. To stop the reaction and fix the tissue, slides were then immersed in 10% sucrose phosphate buffer with 4% Formalin (v/v) for 30 min. Thereafter, slides were dehydrated through a series of alcohols (30, 50, 70, 95% 2×, 100% 2×) and then cleared with xylene and coverslipped with Permount.

Optical densities (OD) of brain and muscle sections and standards were measured using Scion Image (Scion Corp.). Sections were captured on an Olympus B×60 at 4× using SigmaScan Pro v. 3.0 (Jandel Scientific). The system was calibrated using an optical density step tablet (Kodak Calibration Tablet No. 2). Four OD measurements were taken per nucleus on each section, and two to three sections were imaged for a single nucleus. All measurements were taken unilaterally for each subject, and the side of the brain was randomly selected across individuals. On some occasions measurements from the contralateral side were taken because of tissue damage. The experimenter was blind to the treatment of the animals during data acquisition.

Optical density values for each nucleus were then averaged and converted into activity units (μmol/min/g of tissue wet weight) using a regression based on brain homogenate standards included in each batch (Gonzalez-Lima and Cada, 1998). Brain homogenates served as internal calibration standards to control for factors that affect staining intensity. Standards were made by homogenizing whole brains of 12 naïve rats at 4°C, followed by rapid freezing in isopentane. The CO activity of the homogenate was then spectrophotometically assessed (Cada, Gonzalez-Lima, Rose, and Bennet, 1995). Within each reaction, at least two slides with brain homogenates were included, and on each slide were sections cut at varying thickness (10, 20, 30, 40, and 50 μm). The optical densities of these sections were then regressed on the known CO activity of the sections of varying thickness. This regression was used to convert optical density into a standard unit of activity; this allows for the aggregation of data from different batches. The slopes (1093 $\pm$ 53 and 1087 $\pm$ 32) and R^2 values (0.991 and 0.996) for both batches were similar.

Optical density was measured in several limbic brain areas: periventricular preoptic area (PP), rostral medial preoptic area (rMPOA), caudal medial preoptic area (cMPOA), nucleus sphericus (NS), external nucleus of the amygdala (AME), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), lateral septal nucleus (LS), lateral hypothalamus (LH), and habenula (HAB). Brain nuclei were identified using a published atlas (Young, Lopreato, Horan, and Crews, 1994), and alternate sections were counterstained with cresyl violet and used for nucleus identification.

Finally, whole brain metabolism estimates were obtained using an image-processing system consisting of a high-gain camera (Javelin Electronics), a Targa-M8 image capture board, a 486 computer, a Sony color monitor, a DC-powered illuminator, and JAVA software (Jandel Scientific, San Rafael, CA). The system was calibrated using an optical density step tablet (Kodak Calibration Tablet No. 2). This allowed us to measure the OD of entire brain sections, and sections ranging from the torus semicircularis to the striatum were imaged and used in the estimation of whole brain metabolism.

Experimental protocols for both Experiments 1 and 2 adhered to institutional guidelines and *Guidelines for the Use of Animals in Research.*

Statistical Analyses

In Experiment 1, we first assessed whether social housing affected sexual vigor while intact. We analyzed group differences in the proportion of intact males categorized as sexually active (i.e., courted females on at least 50% of screening tests) using a Likelihood Ratio test. Subsequently, we analyzed the proportion of tests in which courtship behaviors were exhibited following castration. Specifically, we analyzed the proportion of tests in which mounts were observed and the proportion in which neck grips were observed, and both were analyzed in the same statistical model using a multivariate analysis of variance (MANOVA). We administered 20 postcastration tests and divided the analysis into two 10-test blocks (GNX1-10 and GNX11-20). Group (HWF or ISOLATE) was sole independent variable, and Behavior was the dependent variable (i.e., proportion of tests with mounts and proportion of tests with neck grips). Proportions were first arc-sine square-root transformed to improve normality. If there was a significant interaction between Group and the dependent variables, we ran separate ANOVAs on each behavior.

We also analyzed the average mount and neck grip latency of individuals who courted at least once (while intact, GNX1-10 and GNX11-20) and both behavioral parameters were analyzed together using a MANOVA. Those that did not court at least once during the experimental period were not included because individuals that did not court were not assigned a latency score.

In Experiment 2, we analyzed group differences using a one-way ANOVA for most brain regions. Each brain area was analyzed separately, and Group was the sole independent parameter. Because three nuclei were measured in the preoptic area (PP, rMPOA, and cMPOA) and two nuclei were measured in the amygdala (NS and AME) we analyzed group differences using a MANOVA with Group as the sole independent variable and Region as the dependent variable.

For all statistical analyses, we set $\alpha = 0.05$. We selected Pillai's trace as our multivariate statistic for all MANOVAs because it is the most robust to deviations from multivariate normality and homogeneity of variance–covariance matrices (Olson, 1974). All analyses were performed using JMP version 3.2 (SAS Institute) for the Macintosh.

RESULTS

Experiment 1: Effects of Housing with Conspecific Females on Courtship Behavior While Intact and Following Castration

There was no difference in the proportion of gonadally intact HWF (10/13 or 77%) and ISOLATE (45/77

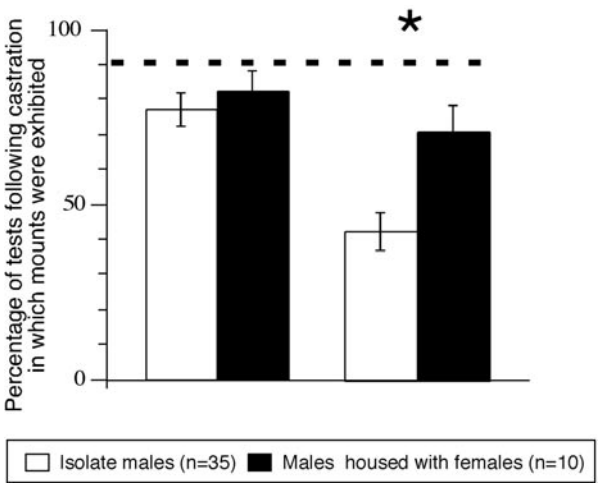

FIG. 1. Differences in mounting frequency following castration between isolate males (n = 35) and males previously housed with females (n = 10). Presented are means ± standard error, and $^*P < 0.05$. The dashed line represents the average proportion of tests in which mounts were shown during the intact screening tests.

or 58%) males that were categorized as sexually active. There was also no difference in courtship latencies between intact HWF and ISOLATE males.

When group differences in courtship frequency during GNX1-10 were analyzed, we found only that the proportion of tests in which mounts were exhibited was significantly greater than the proportion of tests with neck grips (Behavior, $F(1, 44) = 4.8$, $P = 0.034$). When group differences in courtship frequency during GNX11-20 were analyzed, we found that overall HWF males courted females more frequently than ISOLATE males ($F(1, 44) = 5.6$, $P = 0.022$; Fig. 1). The difference is due to the fact that, relative to HWF males, ISOLATE males showed a greater decrement in courtship frequency in GNX11-20 relative to courtship frequency in GNX1-10 (Fig. 1). Though HWF males courted females more frequently, the courtship latencies were not significantly different between HWF and ISOLATE males.

Experiment 2: Effects of Housing with Conspecific Females on Metabolic Capacity (Cytochrome Oxidase Activity) in the Limbic System

Relative to ISOLATE males, HWF males had significantly elevated metabolic capacity in the preoptic area ($F(1, 22) = 6.7$, $P = 0.016$; Fig. 2A) and amygdala ($F(1, 22) = 4.4$, $P = 0.049$; Figs. 2B and 3). Within the preoptic area, metabolic capacity was elevated in the rMPOA and cMPOA relative to the PP (Region, $F(2, 22) = 34.3$, $P < 0.001$). Relative to ISOLATE males (Fig. 4), HWF males also had elevated CO activity in the AH ($F(1, 22) = 9.2$, $P = 0.008$) and VMH ($F(1, 22) = 5.9$, $P = 0.023$). No group difference in CO activity was found in the LS, LH, or HAB (Fig. 4). Estimated whole brain activity and CO activity in hindlimb muscle was not significantly different between HWF and ISOLATE males (Fig. 4). Finally, there was no significant relationship between the sexual vigor of each male displayed during the screening test and CO capacity in any limbic nucleus.

There were no differences in metabolism between HWF males housed with females following hibernation and HWF males housed with females since the previous summer season in hindlimb muscle and all brain nuclei except for the LH ($F(1, 11) = 5.0$, $P = 0.048$). In the LH, males housed with females following hibernation had elevated CO activity.

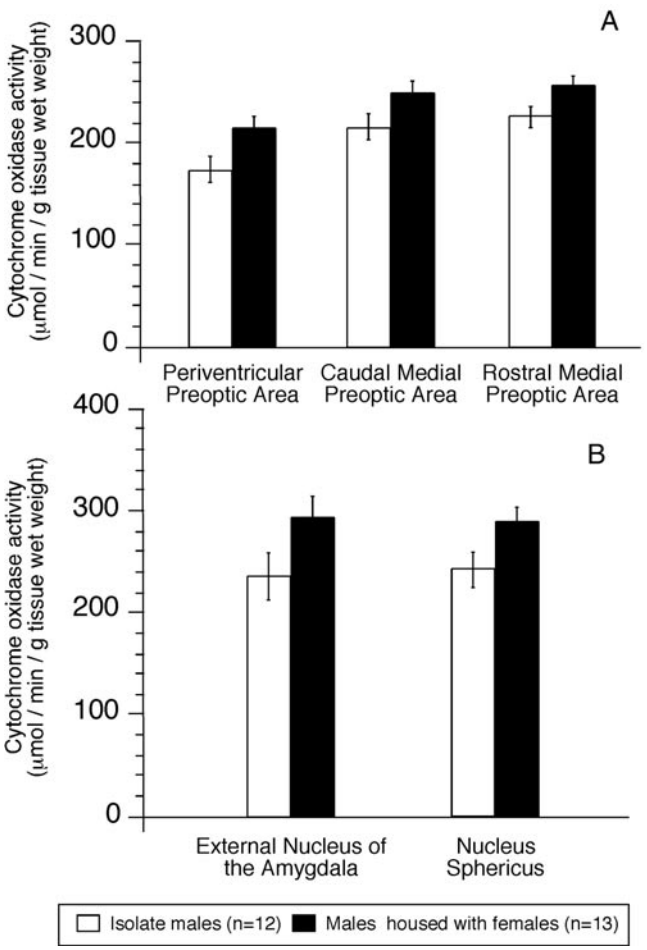

FIG. 2. Differences in metabolic capacity (cytochrome oxidase activity) in the preoptic area (A) and amygdala (B) between isolate males (n = 12) and males housed with females (n = 13). Data were analyzed using a multivariate analysis of variance, and presented are means ± standard error.

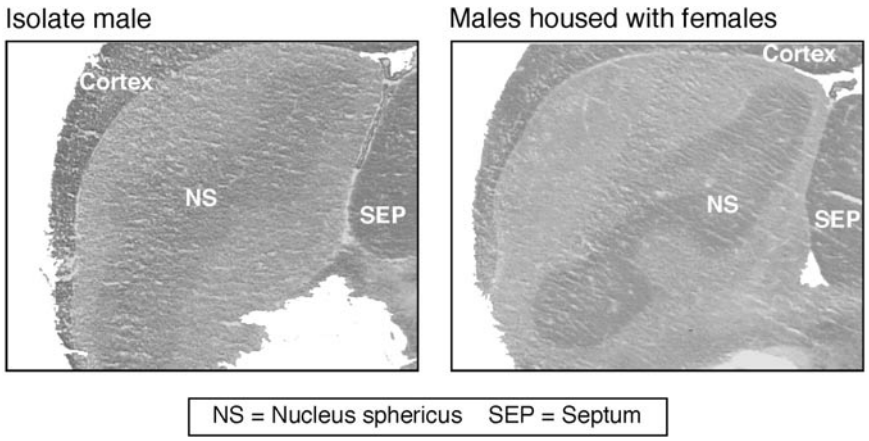

FIG. 3. Representative sections of the nucleus sphericus from males housed in isolation or housed with females. Darker staining means greater cytochrome oxidase activity.

DISCUSSION

Interactions with conspecifics lead to a variety of endocrine and behavioral changes (reviewed in Wallen and Schneider, 2000). For example, in male rodents, copulatory experiences with females lead to short-term elevations in circulating concentrations of androgens (e.g., Kamel, Mock, Wright, and Frankel, 1975) and to increased efficiency in copulation (Dewsbury, 1969; Larsson, 1978; Lumley and Hull, 1999). The retention of sexual behavior following hormone deprivation also increases with copulatory experience: sexually experienced males continue to copulate with females longer following castration than sexually naïve males. This experience-dependent increase in robustness to castration has been documented in male cats (Rosenblatt and Aronson, 1958a,b), rats (Larsson, 1978; Retana-Marquez and Velazquez-Moctezuma, 1997), hamsters (Lisk and Heimann, 1980), and mice (Manning and Thompson, 1976; Phelps *et al.*, 1998).

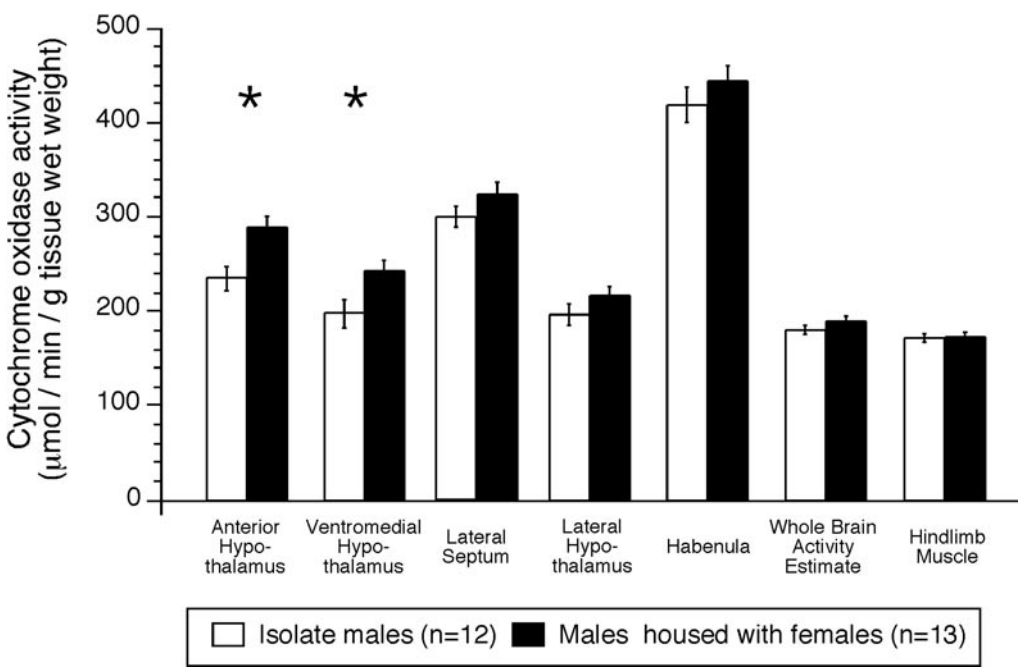

FIG. 4. Differences in cytochrome oxidase activity in other limbic brain areas and hindlimb muscle between isolate males ($n = 12$) and males housed with females ($n = 13$). Presented are means ± standard error, and $^*P < 0.05$.

The effects of housing condition on the retention of courtship behavior following castration in male whiptail lizards (Experiment 1) is reminiscent of the effects of sexual experience on the retention of copulatory behavior in mammals (reviewed in Meisel and Sachs, 1994). We propose that HWF males are more robust to castration than ISOLATE males because they have gained more recent sexual experiences. Though we did not directly quantify the amount of recent sociosexual experience HWF males acquired, females housed with males laid fertile eggs, suggesting that most HWF males were copulating. This is the first study to our knowledge that documents that sociosexual experience while gonadally intact increases the capacity to display courtship behavior following androgen deprivation in male lizards, and this suggests that this type of phenotypic plasticity is not restricted to mammals.

Experiment 1 built upon a previous set of studies in male whiptail lizards which assessed the effect of social stimulation on steroid hormone concentrations in intact males and the effects of social stimulation on courtship behavior in gonadectomized males (Lindzey and Crews, 1988). They reported that HWF males have depressed concentrations of androgens and elevated concentrations of corticosterone and that castrated males housed with females over hibernation showed more courtship behavior upon emergence in the spring. In this study, we report that courtship behavior did not differ between intact HWF and ISOLATE males and that social experience when gonadally intact also increased the propensity to display courtship behaviors in the absence of sex steroid hormones. Taken together, these results show that social experience while gonadally intact depresses androgens without affecting courtship behavior, and social experience, whether acquired when gonadally intact or following castration, increases courtship behavior in the absence of gonadal steroids. The finding that intact HWF males court at the same level as ISOLATE males despite presumably depressed concentrations of androgens is consistent with finding that HWF males court females more frequently following castration: both suggest that courtship behavior is less androgen dependent in HWF males. It is also possible that HWF are overall more sensitive to androgen effects on courtship behavior, and it would be interesting to assess whether HWF males show a quicker reinstatement of courtship behavior following androgen replacement.

The mechanisms underlying changes in the capacity to display copulatory behavior following hormone deprivation are unknown. There are a suite of neural changes following castration that are correlated with the loss of copulatory behavior (reviewed in Meisel and Sachs, 1994). For example, following castration androgen receptor and aromatase concentrations in preoptic, amygdalar, and hypothalamic areas decline (Meisel and Sachs, 1994; Roselli, Stormshak, and Resko, 1998; Lynch and Story, 2000), and somal size, dendritic field, and overall nuclei size decrease in the medial amygdala (e.g., Gomez and Newman, 1991; Cooke, Tabibnia, and Breedlove, 1999). Castration leads to a decrement in the activity of the Na^+, K^+ pump in the preoptic area (Guerra, Rodriguez del Castillo, Battaner, and Mas, 1987) and in CO activity in limbic brain areas (Crews, Coomber, Baldwin, Azad, and Gonzalez-Lima, 1996; Wennstrom, Reeves, and Brenowitz, 2001), suggesting that neural activity decreases following androgen deprivation. Testosterone also increases baseline metabolic activity in the amygdala and hypothalamic areas in male quail (Balthazart, Stamatakis, Bacola, Absil, and Dermon, 2001). Furthermore, the responsiveness of cells in the medial preoptic area to stimulation in the amygdala and lateral septum decreases following castration (Kendrick, 1982, 1983). Overall, it suggests that the integrity of the neural circuits underlying copulation declines following castration. Moreover, it is likely that individual differences in the retention of copulatory behavior following castration are linked to individual differences in the resilience of these neural circuits to castration.

We have previously proposed that experience-dependent increases in metabolic capacity could be, in part, responsible for the enhanced retention of courtship behavior following castration (Sakata *et al.*, 2001). Cytochrome oxidase is a rate-limiting enzyme in ATP synthesis (Wong-Riley, 1989; Gonzalez-Lima, 1992); therefore, the amount of neural activity in particular circuits is constrained by the levels and activity of CO. Further, because the expression of behavior is constrained by neural activity, CO activity could constrain behavioral expression. Because a threshold amount of androgens is required for the activation of male-typical sexual behavior (Beach and Holz-Tucker, 1949; Grunt and Young, 1953; Larsson, 1966) and because androgens stimulate CO activity in limbic brain areas (Crews *et al.*, 1996b), we have proposed that a threshold amount of CO is essential to maintain sufficient neural activity in limbic brain circuits to allow for the expression of copulatory behavior. Cytochrome oxidase activity in brain nuclei critical for male-typical sexual behavior decreases following castration (Crews *et al.*, 1996b), and this may underlie the decrease in courtship behavior following castration.

Finally, we have proposed that factors that elevate CO activity in limbic brain areas will also enhance the retention of sexual behavior following gonadectomy (Sakata *et al.*, 2001).

Here we report that gonadally intact HWF male whiptail lizards have elevated CO activity in limbic brain areas such as the preoptic area and amygdala relative to intact ISOLATE males (Experiment 2) and that HWF males are more likely to court females following castration (Experiment 1). Male rats given daily opportunities to copulate with receptive females have elevated CO activity throughout the vomeronasal circuit (medial amygdala, bed nucleus of the stria terminalis, and medial preoptic area) relative to sexually naive males (Sakata *et al.*, 2002b), and sexual experience in adulthood increases the retention of copulatory behavior following castration in male rats (Larsson, 1978; Retana-Marquez, and Velazquez-Moctezuma, 1997). On the other hand, extensive sociosexual experience in male leopard geckos does not lead to increases in metabolic capacity in preoptic and amygdalar nuclei (Crews *et al.*, 1997) or heightened robustness to castration (Sakata *et al.*, 2002a). Therefore, across three species, experience-dependent increases in robustness to castration are correlated with increased CO activity in preoptic and amygdalar areas.

Differences in CO activity are caused by differences in the metabolic history of brain areas (Wong-Riley, 1989; Gonzalez-Lima, 1992). Consequently, it is likely that increased heterosexual interactions lead to elevated neural activity and, consequently, elevated metabolic capacity in intact HWF males. The MPOA, AH, VMH, AME, and the PP showed increases in metabolic capacity with social housing. These nuclei have been shown to modulate courtship and copulatory behavior in a number of vertebrates (reviewed in Crews and Silver, 1985; Meisel and Sachs, 1994) and to express genes for sex steroid hormone receptors in this species (Young *et al.*, 1994). In male whiptail lizards, MPOA lesions decrease courtship behavior (Kingston and Crews, 1994), whereas hormone implants into the MPOA reinstate courtship behavior (Rozendaal and Crews, 1989; Crews *et al.*, 1996a). Lesions and antagonism of androgen receptors in the VMH significantly inhibit courtship behavior in a variety of species (Farragher and Crews, 1979; Friedman and Crews, 1985; Bernstein, Zuo, and Cheng, 1993; McGinnis, Williams, and Lumia, 1996), and the AH shows heightened neural activity during courtship behavior in male whiptail lizards (Rand and Crews, 1994). In lizards, the AME accumulates androgens (Morrell, Crews, Ballin, Morgentaler, and Pfaff, 1979; Young *et al.*, 1994) and modulates courtship behavior (Greenberg, Scott, and Crews, 1984). The PP has been implicated in the modulation of male-typical courtship behavior in *C. uniparens*, an evolutionary descendant of *C. inornatus* (Godwin and Crews, 1999). Therefore, we propose that these metabolic elevations in HWF are due to increased sociosexual interactions with females.

It is also possible that differences in neurochemical synthesis and/or degradation underlie the behavioral differences found in Experiment 1. For example, it is possible that recent social experience alters the synthesis, release, and/or degradation of dopamine, a neurotransmitter integral in the display of male sexual behavior in rodents (Hull, Du, Lorrain, and Matuszewich, 1997) as well as whiptail lizards (Woolley, Sakata, Gupta, and Crews, 2001). In castrated male rats, there is a positive correlation between whether dopamine is released in the preoptic area when a female is introduced and the probability of copulation (Hull, Du, Lorrain, and Matuszewich, 1995). Similarly, previous social stimulation could facilitate dopamine release in response to females in castrated male whiptail lizards.

We do not know which specific aspects of the social experiences were critical in creating both the behavioral and neural changes. Our experiments only document that the total experience of long-term cohabitation with females produced increases in robustness to castration and neural metabolism. It is possible that the nonsexual aspects of social interaction are responsible for these changes. Furthermore, in Experiment 1, HWF males were administered more screening tests while intact. Because HWF had more screening tests, they could have acquired a stronger conditioning response to contextual aspects of the testing regime (e.g., mice, Kamel *et al.*, 1975; Graham and Desjardins, 1980), and this enhanced conditioning might have contributed to the enhanced display of courtship behavior following castration. We administered an increased number of screening tests to HWF males to augment the prospect of finding group differences in behavior following castration, and we do not feel that this takes away from the conclusion that increased recent sociosexual experience heightens robustness to castration. On the other hand, in Experiment 2, intact HWF and ISOLATE males were both given only five screening tests and were found to differ in neural metabolic capacity. Consequently, we propose that the sociosexual interactions in the home cage were paramount in producing the behavioral and neural differences found in Experiments 1 and 2, respectively.

In these experiments we used field-caught adult animals, thus providing a unique perspective on so-

ciosexual plasticity. First, because animals in this study experienced photothermal conditions similar to those in the field, including a period of winter torpor, our experimental results gain external validity. Second, because male whiptails were field-caught, we do not know the extent to which males in both groups were sexually experienced. Because these males were caught as adults, it is likely that some of the ISOLATE males had sociosexual experiences prior to introduction to the lab. This could be a reason courtship frequency was relatively high even in ISOLATE males 2 weeks following castration (Fig. 1). Consequently, these results suggest that differences in recent sociosexual history even in sexually experienced animals can have significant phenotypic effects. Finally, that we first screened intact animals is important because in studies that document sexual experience effects on postcastration copulatory behavior, the sexual vigor of naïve males is often unknown; in other words, there could have been sexually inactive males in the naïve group. We eliminated the potential confound of group differences in intrinsic sexual vigor by first screening males and castrating only sexually active males (Experiment 1) or by examining groups equal in their sexual vigor (Experiment 2).

In summary, we report that the social plasticity documented in male mammals—increased robustness to castration following sociosexual experience—also exists in male whiptail lizards (Experiment 1). Further, we report that social experience increases metabolic capacity (CO activity) in a suite of limbic brain nuclei in intact males (Experiment 2). Consequently, as in male rats, metabolic increases in the preoptic area and amygdala are correlated with increases in the retention of copulatory behavior following castration in male whiptail lizards.

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