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Differential effects of testosterone and progesterone on the activation and retention of courtship behavior in sexual and parthenogenetic whiptail lizards

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Abstract

Both testosterone (T) and progesterone (P) facilitate the expression of male-typical sexual behavior in a variety of animals, including rodents and lizards. In two species of whiptail lizards, *Cnemidophorus inornatus* and *C. uniparens*, both hormones elicit the full repertoire of courtship behavior. However, the relative efficacy of the two hormones is unknown. In Experiments 1 and 2 we assessed differences in capacity of exogenous T and P to induce male-typical courtship behavior in gonadectomized whiptail lizards. In both species, individuals implanted with T showed more frequent courtship behavior relative to those implanted with P or cholesterol. In Experiments 3 and 4 we examined whether T and P differentially affected the retention of courtship behavior following implant removal. In both species, individuals implanted with T showed more courtship behavior following implant removal than those previously given P. In these experiments, implants were removed at a time when individuals in both groups were behaviorally similar; therefore, the differences in behavior following implant removal were not due to differences in the amount of courtship experience. Taken together, the hormone that was more effective at activating courtship behavior was also more effective at maintaining courtship behavior following implant removal. In summary, though both T and P can elicit identical sexual behaviors in both whiptail species, T has a greater and more lasting effect on courtship behavior and possibly on the neural circuits underlying courtship behavior.

Keywords: Cnemidophorus; Gonadal steroid hormones; Sexual behavior

Introduction

The importance of androgens in the regulation of copulatory behavior has long been established, but the role of progesterone (P) has been studied in only a handful of species. Traditionally, studies have focused on how relatively high doses of P can inhibit the display of male-typical sexual behavior (e.g., Erickson et al., 1967; Erpino, 1973; Morin, 1977). However, recent studies in rats, mice, and lizards highlight the facilitatory role of P in the expression of copulatory behavior in males (reviewed in Crews and Sakata, 2000). For example, P can induce mounting behavior in gonadectomized male rats (Witt et al., 1995), and male mice lacking a functional progesterone receptor show deficits in mounting behavior during their first copulatory experience relative to wild-type males (Phelps et al., 1998). Although it has become evident that both androgens and progestins can activate copulatory behavior, their potencies have not been compared.

A common way to study the efficacy of a sex steroid hormone is to assess its capacity to induce the expression of social behaviors; this method was utilized in the classic studies that led to the aromatization hypothesis (reviewed in Meisel and Sachs, 1994). In general, hormones that activate copulatory behavior more quickly or in a greater proportion of individuals are considered to be more potent activators of

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copulation. For example, whereas dihydrotestosterone does not readily activate copulatory behavior in castrated male rats, estradiol is effective at reinstating copulatory behavior in castrated males (Meisel and Sachs, 1994). Because gonadal steroid hormones activate sexual behavior in rodents by changing the vomeronasal circuit [accessory olfactory bulb, medial amygdala (MeA), bed nucleus of the stria terminalis (BNST), and medial preoptic area (MPOA)] such that copulatory behavior is expressed when females are presented (Meisel and Sachs, 1994), it is plausible that more potent hormones have greater effects on the "strength" and/or responsiveness of this circuit.

We propose that another way to assess the potency of steroid hormones with regard to male-typical sexual behavior is to assess how long copulatory behavior persists following hormone deprivation. Following androgen deprivation, copulatory behavior gradually disappears, and this loss is correlated with a suite of neural changes that are likely to be causally related to the decline in sexual behavior (Meisel and Sachs, 1994; Crews et al., 1996a; reviewed in Kendrick, 2002). For example, the number of cells in the BNST responding to antidromic stimulation in the MPOA decreases following castration in male rats (Kendrick, 1982), suggesting that the functional connectivity between the BNST and the MPOA decreases following androgen withdrawal. The resilience of pertinent neural parameters to hormone deprivation is likely to be related to the rate at which sexual behavior declines following hormone deprivation; thus, individuals with "stronger" or more resilient circuits are likely to show a slower decline in sexual behavior. It follows that hormones with different effects on the "strength" of the neural copulatory circuit could have different effects on the retention of copulatory behavior following hormone deprivation.

Cnemidophorus lizards provide a useful model system in which to compare the potencies of T and P because both hormones can elicit the full repertoire of courtship behaviors (reviewed in Crews and Sakata, 2000). In male *C. inornatus*, androgens fluctuate seasonally in parallel with courtship behavior, but P concentrations remain low across seasons (Moore and Crews, 1986). However, following castration, administration of P or T can reinstate sexual behavior in a subset of males (Lindzey and Crews, 1986, 1988), and although P is a precursor to T, it has been established that P acts via its own receptor (Lindzey and Crews, 1988). A related whiptail lizard, *C. uniparens*, is a triploid, parthenogenetic species that evolved through two hybridization events, both involving *C. inornatus* (Crews and Sakata, 2000). These parthenogens show both female-like and male-like sexual behavior depending on their follicular state (Moore et al., 1985a). Parthenogens display female-like receptive behavior when estradiol concentrations are high (when follicles are large) and male-like pseudocopulatory behavior when P concentrations are high (following ovulation). Androgen concentrations remain undetectable throughout the cycle (Moore et al., 1985b), suggesting that P is paramount in eliciting this behavior in intact parthenogens, but administration of either P or T induces pseudocopulatory behavior in ovariectomized individuals (Grassman and Crews, 1986; Mayo and Crews, 1987; Wade et al., 1993). Finally, it is hypothesized that P-dependent activation of male-like courtship behavior in the parthenogen was inherited from *C. inornatus* (Crews, 1989).

In this series of experiments, we compared the differential effects of T and P on the activation of courtship behavior and on the retention of courtship behavior following hormone deprivation in gonadectomized *C. inornatus* males and *C. uniparens* parthenogens. We assessed the frequency of courtship behavior of animals given T or P both when implanted with hormones and following implant removal. We found that, relative to P, T had greater effects on both the activation and the retention of courtship behavior in both species, suggesting that T might have greater effects on the neural substrate underlying courtship behavior.

Materials and methods

Animals

Cnemidophorus inornatus males were collected near Sanderson, Texas, in the summer of 1999 under permission from the State of Texas. Animals were transported to the lab then housed individually ($25 \times 32 \times 32$ cm). *Cnemidophorus uniparens* were collected near Portal, Arizona, and Rodeo, New Mexico, in the summers of 1999 (Experiment 2) and 2001 (Experiment 4) under permits from Arizona and New Mexico, and individuals were housed in groups of 4–5 ($75 \times 32 \times 32$ cm) upon arrival to the lab. All animals were kept on a 14:10 L:D cycle with temperatures ranging from 23 to 33°C. They were fed crickets or mealworms dusted with supplemental vitamins (1:1 mix of Herptivite and phosphorus-free calcium with vitamin D_3 (Rep-Cal Research Labs, CA)) three times a week, and water was provided ad libitum.

Behavioral screening for C. inornatus

After a 1-week habituation period, all *C. inornatus* males were given 10 daily tests to screen for sexual vigor by placing a receptive female into the male's home cage. Wood blocks and water dishes were removed from individual cages at least 10 min before testing, and all tests were conducted between 10 AM and 2 PM, the active period for this species. All stimulus females were screened for receptivity using a sexually vigorous male prior to use. Courtship behavior is virtually identical between *C. inornatus* males and the parthenogen and has previously been described in these species (Lindzey and Crews, 1986). Briefly, individuals will approach, mount, and then neck grip the stimulus female. These behaviors are hierarchical; for example, neck

gripping does not occur without mounting. One to 3 min after mounting, sexually vigorous animals will attempt to intromit.

For all behavior tests using *C. inornatus* males, tests were stopped after 3 min if a male failed to court the female or before a male attempted to intromit, whichever came first. Tests were stopped before intromission to minimize the amount of sexual experience gained during the tests. 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 et al., 1996b; Sakata et al., 2002b), 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). Males that mounted on at least 50% of their screening tests were considered to be sexually active (e.g., 3 out of 5 consecutive tests), a criterion that has been used in past studies (Lindzey and Crews, 1986, 1988, 1992a,b; Sakata et al., 2002b). Approximately 60% of the males brought into the laboratory were categorized as sexually active using this criterion, and this is consistent with previous observations (Lindzey and Crews, 1992a).

After the screening tests, sexually active males were gonadectomized under cold anesthesia and subsequently screened for sexual behavior. After males reached a criterion for loss of behavior (i.e., 5 consecutive tests without a mount) they were given an intraperitoneal Silastic implant filled with P (12 × 1.47 mm i.d. × 1.96 mm o.d.). Males were allowed 3 days to recover from the surgery and then were tested daily for 20 consecutive days. A male was considered P-sensitive if he courted on any 3 tests out of 5 consecutive tests; in other words, if at any time he reached the criterion for sexual activity (see above) he was considered P-sensitive. For example, if a P-implanted male courted the stimulus females on tests 4, 6, 10, 11, and 13, he would be considered to be sexually active because he courted on 3 occasions between tests 9 and 13. Monitoring a male's behavior in this manner allowed us to determine when males became sexually active. As documented in previous studies, we found that ~70% of sexually active males were P-sensitive (Lindzey and Crews, 1992b).

There are individual differences in the sensitivity to P in *C. inornatus* males (Lindzey and Crews, 1992b). In order to appropriately compare the effects of T and P, it is important to control for the sensitivity to P; otherwise, differences in the effects of T vs P could be due to individual differences in the capacity for P to elicit courtship across groups. Therefore, only P-sensitive males were used, and P-sensitive males were pseudorandomly allocated into groups such that the groups were equal in the frequency of courtship behavior exhibited during the P-sensitivity screening tests. Because recent sociosexual experience can affect the expression of courtship behavior in this species (Sakata et al., 2002b), this allocation also ensured that groups were comparable in their recent experiences. Furthermore, because P-sensitive males are putatively involved in the hybridization process leading to the evolution of the parthenogen (Crews, 1989), species comparisons are more valid between P-sensitive (versus P-insensitive) *C. inornatus* and *C. uniparens*.

This type of behavioral screening process did not occur for *C. uniparens*, and the specifics for each experiment are presented below.

Surgeries and hormone manipulations

All surgeries were done under cold anesthesia, and all implants were 12 × 1.47 mm i.d. × 1.96 mm o.d and given intraperitoneally. This implant sizes for P and T have previously been found to successfully elicit courtship behavior in both species (Grassman and Crews, 1986; Lindzey and Crews, 1986, 1988; Wade et al., 1993; Crews et al., 1996b) and have been reported to produce P concentrations ranging from 36 ± 11 (Lindzey and Crews, 1986) to 80 ± 11 ng/ml (Lindzey and Crews, 1988) and T concentrations of 87 ± 17 ng/ml (Lindzey and Crews, 1986). Therefore, the range of concentrations of each hormone is comparable, though slightly higher for T.

Because P is a biosynthetic precursor to T, it can be argued that the differences in the effects of P and T are due to the fact that only some of the P is being metabolized into T. Though we cannot rule this out, there is evidence that P acts via its native receptor to induce courtship behavior in whiptail lizards (Lindzey and Crews, 1988). Furthermore, we anticipate that this contribution is relatively minor because P implants do not significantly increase circulating concentrations of T in castrated animals (Lindzey and Crews, 1988).

All experimental protocols were approved by the Institute for Animal Care and Use Committee of the University of Texas at Austin and adhered to the *Guidelines for the Use of Animals in Research*.

Experiment 1: The effects of testosterone, progesterone, and cholesterol on the expression of courtship behavior in gonadectomized male C. inornatus

Males identified as P-sensitive had their P implants removed and simultaneously received a Silastic implant filled with T ($n = 8$), P ($n = 8$), or cholesterol (CHOL; $n = 9$). Beginning 3 days after implantation, we administered 10 daily tests with receptive females. Two males given T implants were tested only 8 times. Testing protocol was similar to tests administered during the screening processes.

We assessed the effect of T, P, and CHOL (Hormone) on the proportion of tests in which mount, neck grip, and intromission behaviors were displayed using a multivariate analysis of variance (MANOVA). In other words, Hormone was the sole independent parameter, and the proportions for the 3 behaviors were the dependent variables (Behavior). Behavior was analyzed in this multivariate way to assess how each steroid hormone affects courtship as a whole. If

there was a significant effect of Hormone, multivariate contrasts were performed, and we set $\alpha = 0.013$ (Bonferroni correction for 3 contrasts). If there was a significant interaction between Hormone and Behavior, we conducted separate univariate ANOVAs for each behavior.

Proportion data were square-root arc-sine transformed to improve normality (Stevens, 1996), and we selected Pillai's trace as our multivariate statistic because it is the most robust to deviations from multivariate normality and homogeneity of variance-covariance matrices (Olson, 1974). This is true for statistical analyses in all experiments reported here. Unless otherwise noted, we set $\alpha = 0.05$ for all statistical analyses. All analyses were performed using JMP version 3.2 (SAS Institute) for the Macintosh.

Results

Castrated males implanted with different hormones differed significantly in the proportion of tests in which courtship behavior was displayed ($F_{2,22} = 6.6$, $P = 0.006$; Fig. 1a). Males implanted with T courted females significantly more frequently than males implanted with CHOL ($P = 0.002$). Males implanted with T courted more frequently than males given P, but this difference was not significant after correction for multiple contrasts ($P = 0.026$). Males implanted with P and CHOL did not differ in courtship frequency. Mounts, neck grips, and intromissions were exhibited at equal frequencies.

Experiment 2: The effects of testosterone, progesterone, and cholesterol on the activation of pseudosexual behavior in gonadectomized parthenogens, C. uniparens

Group-housed parthenogens that were reproductively cycling were ovariectomized and then housed individually (25 × 32 × 32 cm). Individuals were left alone for at least a week to allow for clearing of endogenous sex steroid hormones before receiving a Silastic implant containing T ($n = 9$), P ($n = 9$), or CHOL ($n = 7$). The parthenogens were not first screened for P-sensitivity because they are clonal and are all assumed to be similar in P-sensitivity. Beginning 3 days after implantation, individuals were tested daily for 10 consecutive days for courtship behavior with a sexually receptive parthenogen. Tests lasted for 60 min and were stopped when the individual attempted to "donut" (pseudocopulate) the stimulus animal. The data were analyzed just as in Experiment 1.

Results

Gonadectomized parthenogens implanted with different hormones differed significantly in the proportion of tests in which courtship behavior was displayed ($F_{2,22} = 17.5$, $P < 0.001$; Fig. 1b). Parthenogens implanted with T courted stimulus females significantly more frequently than parthenogens implanted with CHOL ($P < 0.001$) or with P ($P < 0.001$). Parthenogens implanted with CHOL or P showed minimal sexual behavior. There was also an effect of Behavior ($F_{1,22} = 4.5$, $P = 0.024$), with mounting behavior being exhibited more often than both neck grips and intromissions.

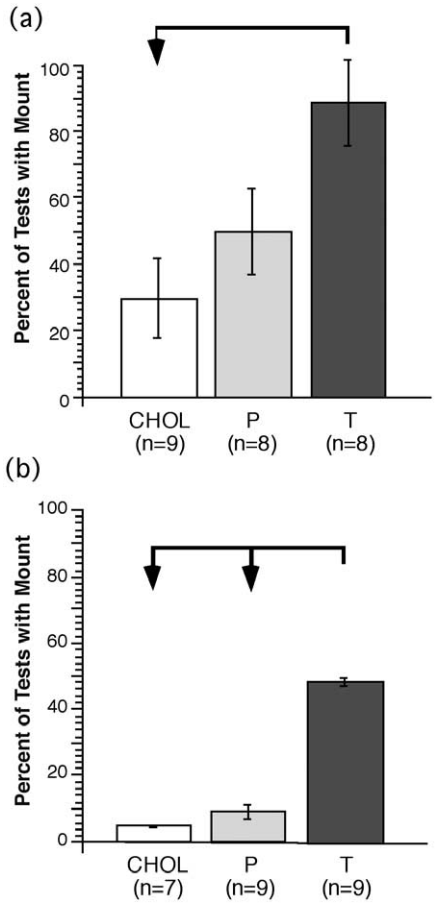

Fig. 1. Effects of cholesterol (CHOL), progesterone (P), and testosterone (T) on the percentage of tests in which mounting behavior was displayed in (a) castrated *Cnemidophorus inornatus* males and (b) ovariectomized *C. uniparens*. Significant differences are denoted by arrows ($\alpha = 0.013$, Bonferroni correction), and sample sizes are indicated in parentheses. Presented are means ± SEM.

Experiment 3: The differential effects of testosterone and progesterone on the retention of courtship behavior in gonadectomized male C. inornatus

Progesterone-sensitive males were pseudorandomly distributed into two groups such that the groups were equal in the amount of courtship exhibited during the P-sensitivity screening tests. Progesterone implants were removed, and males were given a new implant of either T ($n = 13$) or P ($n = 11$). Beginning 3 days after reimplantation males were given daily tests with receptive females using the same protocol used during screening tests. Males were tested until they courted females on at least 3 out of 5 consecutive tests (criterion for sexual activity), and all males reached this

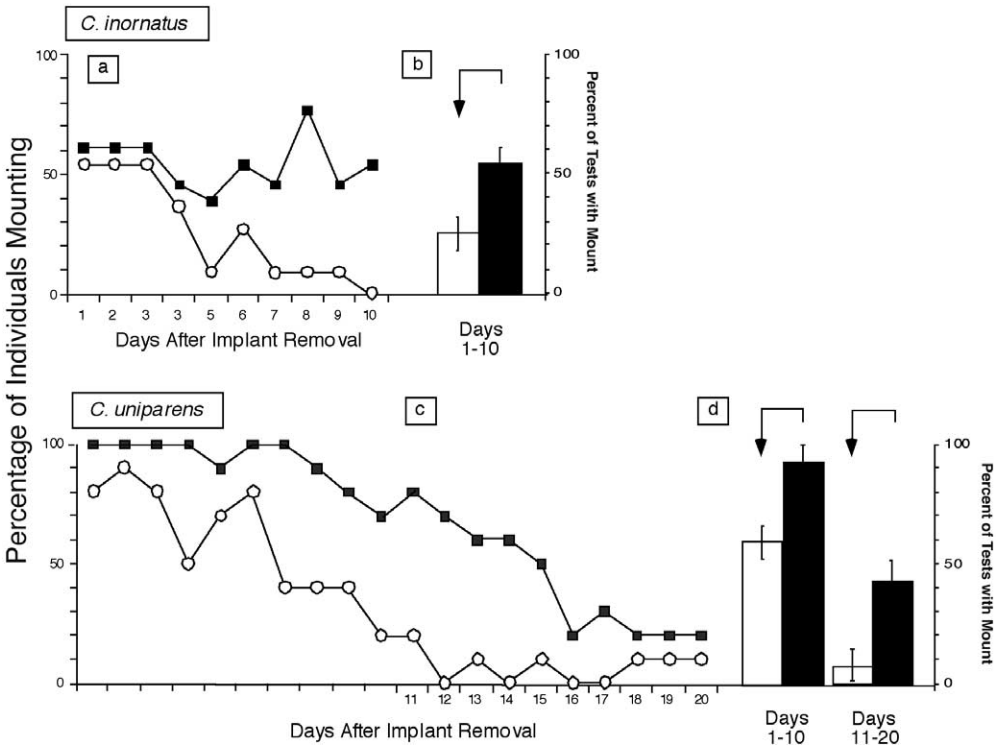

Fig. 2. Display of mounting behavior following the removal of T and P implants in (a) castrated *C. inornatus* males and (b) ovariectomized *C. uniparens*. The left of each display depicts the decline in mounting behavior with time; plotted is the proportion of males in each group that mounted on a particular test day following implant removal. This highlights the facts that all groups show a decline in mounting behavior following implant removal and, moreover, that the decline is slower in the individuals previously implanted with T. On the right is the average percentage of tests in which mounts are displayed for each group. Presented are means ± SEM, and only these data are analyzed here. Significant differences are denoted by arrows, and sample sizes are indicated in parentheses.

criterion between 5 and 7 tests. On the day after the males reached this criterion, implants were removed, and beginning the day after surgery, males were given 10 daily tests with a receptive female. These tests were stopped after the male neck-gripped the female.

We analyzed differences in the proportion of tests in which courtship was exhibited following implant removal between male *C. inornatus* previously given T or P. The same statistical model was used as in Experiment 1.

Results

There was a significant interaction between Hormone and Behavior on courtship behavior following implant removal ($F_{1,22} = 8.9$, $P = 0.007$). Consequently, we analyzed group differences in mounting and neck-gripping behavior separately. We found that males previously implanted with T mounted females on more tests following implant removal than males previously given P ($F_{1,22} = 7.6$, $P = 0.012$; Fig. 2b); however, we found no difference in neck-gripping behavior. This difference in mounting behavior is due to a slower decline in mounting behavior in males previously given T (Fig. 2a). In the main analysis, the effect of Behavior was also significant ($F_{1,22} = 31.2$, $P < 0.001$), with mount frequency being greater than that of neck grips.

Experiment 4: The differential effects of testosterone and progesterone on the retention of courtship behavior in gonadectomized parthenogens, C. uniparens

Group-housed parthenogens that were reproductively cycling ($n = 30$) were ovariectomized and simultaneously given an implant of T. Following surgery, all parthenogens were housed individually (25 × 32 × 32 cm). Beginning 3 days after implantation, all parthenogens were tested daily with receptive conspecifics, and 17–18 tests were administered. Tests were stopped after 15 min or when the individual tried to pseudocopulate. We screened ovariectomized parthenogens for sexual activity using T implants instead of P implants because P did not elicit robust pseudocopulatory behavior in the 1-h tests (Experiment 2). Whereas using P implants would have made this experiment comparable to Experiment 3, we would have needed to use longer tests and many more experimental animals in order to get a sufficient number of sexually vigorous parthenogens.

Only individuals that attempted to pseudocopulate with the stimulus female on at least 3 out of any 5 consecutive tests (criterion for sexual activity) were used in the next experimental phase ($n = 20$). On average, these parthenogens mounted stimulus females on 70% of tests. Three or 4 days after their last screening test, T implants were removed

and replaced with a new T ($n = 10$) or P ($n = 10$) implant. The groups were balanced such that the frequency of courtship behavior exhibited during the screening phase was equal. Individuals were given 3 days to recover and then were administered 5 daily tests using the same behavioral protocol as before. All P- and T-implanted parthenogens continued to show pseudocopulatory behavior with their new implants, and all individuals mounted stimulus females immediately upon their introduction. After these 5 tests, the implants were removed, and the animals were given 20 daily tests with receptive females beginning the day after implant removal. This time, experimental animals were given only 3 min to mount the receptive stimulus female, and if they mounted they were given an additional 5 min from the time of mount to attempt to pseudocopulate. We gave them less time to mount stimulus females in order to make the protocol similar to that given to *C. inornatus* males in their postimplant removal tests (Experiment 3). As with previous tests, tests were stopped when the individual attempted to pseudocopulate.

We analyzed differences in the proportion of tests in which courtship behavior was exhibited following implant removal between parthenogens previously given T or P. We divided the 20 postimplant removal tests into two 10 test blocks (POST1–10 and POST11–20). We analyzed group differences using a two-way repeated-measures MANOVA with Hormone (T or P) and Time (POST1–10 and POST11–20) as the independent variables, and Behavior (mount and intromission) as the dependent variable. We also added ID as a random variable nested within Hormone group; adding this factor eliminates the variability among subjects due to individual differences from the error term (Sokal and Rohlf, 1995; Stevens, 1996).

Results

Following the removal of the second implant (P or T), parthenogens previously implanted with T showed more frequent pseudocopulatory behavior than those previously given P ($F_{1,34} = 7.56$, $P = 0.010$; Fig. 2d). This was due to a slower rate of decline in courtship behavior in parthenogens previously given T (Fig. 2c). Courtship frequency was also affected by Time ($F_{1,34} = 52.9$, $P < 0.001$), with frequency being lower at POST11–20 than at POST1–10 (Fig. 2d).

Discussion

Sex steroid hormones differ in their capacity to induce male-typical sexual behavior. For example, in male rats, dihydrotestosterone does not significantly activate copulatory behavior whereas estradiol does (reviewed in Meisel and Sachs, 1994). In whiptail lizards, both testosterone (T) and progesterone (P) can activate courtship behavior, but here we report that gonadectomized *C. inornatus* and *C. uniparens* implanted with T exhibited more frequent courtship behavior relative to those implanted with P or cholesterol (CHOL) (Experiments 1 and 2). We also found that gonadectomized whiptails of both species implanted with T continued to show courtship behavior following implant removal longer than those previously implanted with P (Experiments 3 and 4). Because the amount of courtship experience during the screening processes was equal across males given T or P, differences in behavior following implant removal were not due to differences in social experience. Taken together, the hormone that more effectively induced courtship behavior also increased the retention of courtship behavior following hormone deprivation.

This is the first study, to our knowledge, that has compared the effects of different sex steroid hormones on both the activation of courtship behavior following gonadectomy and retention of courtship behavior following implant removal. Experiments 1 and 2 are analogous to classic studies leading to the aromatization hypothesis (reviewed in Meisel and Sachs, 1994) and based on the notion that hormones that are more effective at inducing copulatory behavior are likely to have greater effects on the "strength" of the neural circuit controlling sexual behavior. Experiments 3 and 4 were based on the notion that the "stronger" the neural circuit underlying male-typical sexual behavior the longer sexual behavior will be retained following hormone deprivation (Sakata et al., 2001). It has been proposed that sociosexual experience can increase the "strength" or resilience of the neural circuit underlying copulatory behavior (Rosenblatt and Aronson, 1958a; Sakata et al., 2001), and interestingly, the behavioral differences between males given T or P are similar to differences between sexually experienced and naïve males. Like experienced males, whiptail lizards given T show quicker activation of courtship behavior following hormone administration and slower decline in sexual behavior following hormone deprivation (Rosenblatt and Aronson, 1958a,b; Manning and Thompson, 1976; Larsson, 1978; Lisk and Heimann, 1980; Retana-Marquez and Velazquez-Moctezuma, 1997; Phelps et al., 1998; Sakata et al., 2002a,b). It would be interesting to assess whether neural phenotypes that are differentially affected by T or P are those that are affected by experience.

Neural parameters that fluctuate with the expression of sexual behavior might lend insight into the "strength" of the neural circuit, and moreover, it would be interesting to assess whether T and P differentially affect these parameters. For example, the display of copulatory behavior is correlated with lower refractory periods of amygdalar neurons projecting to the MPOA (Kendrick, 1981; reviewed in Kendrick, 2002); castration increases and androgen treatment depresses their refractory periods. It is possible that T more quickly depresses the refractory periods of amygdalar neurons relative to P and that amygdalar neurons show slower increases in refractory periods following implant removal in individuals previously given T. The metabolic capacity and androgen receptor immunoreactivity in and the

size of the MPOA and MeA decrease following castration and increase following androgen replacement (Crews et al., 1996a; Cooke et al., 1999; Lynch and Story, 2000). It would be interesting to test whether decreases in these parameters following hormone deprivation are slower in individuals previously given T than P and whether increases in these parameters are quicker following T administration than P administration. Because dopamine agonists induce mounting behavior in whiptail lizards (Woolley et al., 2001), T and P might also differentially affect limbic dopaminergic systems. Finally, because peripheral factors could contribute to behavioral differences, it is possible that differences in clearance rates could exist between T and P (e.g., T could take longer to metabolize therefore those previously given T continue to court longer). However, we argue that this is not likely to be a major factor because androgen concentrations fall to undetectable levels within hours following castration in male rats (Keating and Tcholakian, 1983; Liebmann and Matsumoto, 1990), and behavioral differences between whiptail lizards previously given T and P emerged many days following implant removal (Fig. 2). Therefore, it is likely that both hormones fall to undetectable levels well before these behavioral differences emerge.

The finding that implantation with T can lead to an enhanced retention of courtship behavior following implant removal can also be seen when we compare the behavior of *C. inornatus* males to that of the parthenogen (Experiments 3 and 4). Overall, male *C. inornatus* showed less frequent mounting behavior during the 10 tests following implant removal relative to *C. uniparens* (Fig. 2). Male *C. inornatus* were first screened for P-sensitivity and then subsequently given a T or P implant; in other words, these males were stimulated with androgens for at most 1 week. The parthenogens, on the other hand, were first screened with T and then given a subsequent P or T implant. Those that received a new T implant after screening had approximately 4 weeks of androgen exposure, and those that received a P implant following screening had 3 weeks of androgen exposure followed by 1 week of P stimulation. Taken together, across the species, there is a correlation between the total duration of androgen exposure and the frequency of courtship behavior following implant removal. We do not attribute this difference to intrinsic species differences because, on average, the parthenogens are less sexually vigorous than *C. inornatus* males.

We found that the capacity for P to activate sexual behavior depends, to an extent, on prior hormonal and behavioral experience. For example, in Experiment 1, P-implanted *C. inornatus* did not exhibit much courtship. This was surprising because most of these individuals showed robust courtship behavior during the P-screening tests. It is possible that, over time, P reduces the concentration of its receptor in critical brain nuclei, making the individual less sensitive to the effects of P with time. Grassman and Crews (1986) gave parthenogens P implants at the time of ovariectomy and found that P induces pseudocopulatory behavior in parthenogens, whereas we administered P implants at least 1 week following ovariectomy and did not find significant induction of pseudocopulatory behavior (Experiment 2). This difference in recent hormonal experience could have contributed to discrepancy in our results (i.e., activation vs maintenance of sexual behavior). Interestingly, after nearly 3 weeks of training with T implants, parthenogens given P courted stimulus females readily and quickly within 15 min (Experiment 4). This increase in courtship propensity could be due to enhanced sensitivity to P due to androgenic stimulation, since T increases the expression of P receptor mRNA in the periventricular preoptic area (Godwin et al., 2000), and/or to courtship experience (Meisel and Sachs, 1994; reviewed in Wallen and Schneider, 2000; Pfaus et al., 2001; Sakata et al., 2002a). Decreased courtship latencies with repeated testing were found in T-implanted parthenogens in Experiments 2 and 4 (data not shown), and this training could have facilitated courtship behavior when given the new P implant in Experiment 4. Finally, the fact that pseudocopulatory behavior is reliably observed in intact, gravid, group-housed females in our lab suggests that other hormonal factors (such as estrogen priming) or additional social stimuli (group housing) facilitate the expression of this behavior in the parthenogen.

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