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# Testosterone, Paternal Behavior, and Aggression in the Monogamous California Mouse (*Peromyscus californicus*)

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**Testosterone (T) mediates a trade-off, or negative correlation, between paternal behavior and aggression in several seasonally breeding avian species. However, the presence or absence of a T-mediated trade-off in mammals has received less attention. We examined the relationship between paternal behavior and territorial aggression in the biparental California mouse, *Peromyscus californicus.* In contrast to seasonally breeding birds, T maintains paternal behavior in this year-round territorial species. Castration reduced paternal behavior, whereas T replacement maintained high levels of paternal behavior. We hypothesize that T is aromatized in the brain to estradiol, which in turn stimulates paternal behavior. In contrast to paternal behavior, aggressive behavior was not reduced by castration. Interestingly, only sham males showed an increase in aggression across three aggression tests, while no change was detected in castrated or T-replacement males. Overall, trade-offs between aggression and paternal behavior do not appear to occur in this species. Measures of paternal behavior and aggression in a correlational experiment were actually positively correlated. Our data suggest that it may be worth reexamining the role that T plays in regulating mammalian paternal behavior.** © 2001 Academic Press

***Key Words:*** **testosterone; aggression; paternal behavior; trade-offs; *Peromyscus californicus.***

Theoretically, males should increase their reproductive success by mating with as many females as possible while providing little paternal care (Trivers, 1972). However, there are many exceptional cases, as males provide paternal care in a diversity of taxa (fish, Gross and Sargent, 1985; birds, Ketterson and Nolan, 1994; rodents, Dewsbury, 1985; primates, Snowdon, 1996). Over the past two decades, researchers have begun to focus on the importance of male paternal behavior. One intriguing finding, primarily in seasonally breeding birds, is that there is evidence for a trade-off between paternal behavior and aggression (Hegner and Wingfield, 1987; Ketterson and Nolan, 1992). Males that spend more time engaged in aggressive territorial activity spend less time feeding offspring. In these species, males tend to have increased baseline testosterone (T) levels during the spring and summer breeding season, which then decline when they provide paternal care. Studies of paternal behavior and the trade-off hypothesis in mammals have been somewhat limited, as paternal behavior is observed in only 5% of all mammalian species (Clutton-Brock, 1991).

There are several behavioral and physiological differences between birds and mammals that could affect the applicability of the trade-off model. In rodents, paternal behavior consists of behaviors such as thermoregulatory behavior (huddling), grooming pups, and retrieving pups to the nest. In contrast to male birds that must leave the nest to forage for their offspring, most rodent paternal behaviors do not require a male to leave the immediate area of the nest. More importantly, many paternal mammalian species have a postpartum estrus (Dietz, Baker, and Miglioretti, 1994; Gubernick, 1988; Parkening and Collins, 1991; Ziegler, Bridson, Snowdon, and Eman, 1987), so that males must also be ready to mate at the same time when paternal care is required. If males guard their mates from other males (i.e., Gubernick and Nordby, 1993), high levels of aggressive behavior may also be favored immediately after parturition, when the female is fertile. In species in which males must show paternal behavior and aggression simultaneously

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there may not be a trade-off, and there may be plasticity in how T modulates these behaviors.

Many studies have investigated the effects of T on behavior in nonpaternal mammals. In a variety of mammals, including rodents, T promotes male sexual behavior (see Baum, 1992 for review). While castration reduces aggression in golden hamsters (Vandenbergh, 1971), domestic mice (Leshner and Moyer, 1975), and rats (Albert, Walsh, Gorzalka, Siemens, and Louie, 1986), there is some variation in how baseline T levels affect aggression. Castrated male prairie voles (Demas, Moffatt, Drazen, and Nelson, 1999) and wood rats (Caldwell, Glickman, and Smith, 1984) are as aggressive as intact males. These data from prairie voles and wood rats suggest that there may be some variation in how T influences aggression. In the CF-1 stock of domestic mice, castration reduces aggression toward pups, and T replacement increases the proportion of males that are infanticidal (Perrigo, Bryant, and vom Saal, 1989). In addition to the many studies investigating the relationships between T and aggression, there has also been interest in how T influences paternal care.

In general, fewer manipulative studies have examined how T modulates paternal behavior in mammals. Correlational studies in rodents have found an increase in circulating male T levels just prior to parturition, followed by a decrease in T following the birth of pups (Mongolian gerbils, Brown, Murdoch, Murphy, and Moger, 1995; Djungarian hamsters, Reburn and Wynne-Edwards, 1999). In male cotton-top tamarins, male urinary T levels gradually increase during pregnancy and remain elevated after parturition (Ziegler and Snowdon, 2000). Postnatal injections of T (1–6 days postpartum) reduce alloparental behavior in juvenile male prairie voles (Roberts, Zullo, Gustafson, and Carter, 1996). In adult male prairie voles, the effect of castration on paternal behavior has been inconsistent, with one study reporting a decrease in paternal behavior (Wang and De Vries, 1993), and a second reporting no effect (Lonstein and De Vries, 1999). Castrated male gerbils were found to show higher levels of paternal behavior than sham males or castrated males with T implants (Clark and Galef, 1999). Based on these data, it is difficult to frame a general explanation for how circulating T is related to paternal behavior.

An attractive model system for studying the effect of T on aggression and paternal behavior is the biparental California mouse, *Peromyscus californicus*. Males and females form pair bonds and cohabitate for prolonged periods of time (McCabe and Blanchard, 1950; Ribble and Salvioni, 1990). In contrast to socially monogamous species that engage in extrapair copulations (rock-wallaby, Spencer, Horsup, and Marsh, 1998; indigo bunting, Westneat, 1990), DNA paternity analysis indicates that California mice form exclusive mating pairs (Ribble, 1991). Paternal behavior is an important aspect of this species' life history. When the father is removed, offspring survival is reduced under cold climate conditions both in the laboratory and in the field (Gubernick, Wright, and Brown, 1993; Gubernick and Teferi, 2000). One study found a nonsignificant trend for fathers (which provide more paternal care) to have lower T levels than nonfathers (Gubernick and Nelson, 1989). It is unknown how aggression and paternal behavior are related to one another in this species or whether T is important in mediating these behaviors.

The experiments described below address two major questions. First, aggression and paternal behavior are assessed within individuals to determine whether there is a relationship between these behavioral classes. A negative correlation between aggression and paternal behavior would suggest that there is a trade-off between aggression and paternal behavior. No correlation or a positive correlation between aggression and paternal behavior would suggest the absence of a trade-off. Second, the role of T is experimentally examined in the expression of paternal behavior and aggression. Other correlational research suggests that T may be negatively associated with paternal behavior in rodents, and most experimental research suggests that castration should reduce aggressive behavior.

## METHODS

### *Subjects*

We used reproductively experienced male mice reared in a laboratory colony at the University of Wisconsin, Madison. Subjects were housed in male–female pairs in standard cages and were fed Purina 5001 mouse chow and water *ad libitum*. Colony rooms were kept under a 13L:11D light cycle with lights on at 0500. Behavioral observations were conducted during the dark phase between 1900 and 2100 under dim red light. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. No siblings were used within any of the three experiments described below.

### Correlational Study

To investigate the relationship between paternal behavior and aggression in California mice, we assessed 31 males for paternal behavior and aggression on consecutive nights. One day after a litter was born, we placed the father, mother, and pups into a large Plexiglas observation cage. The cage contained one smaller chamber (22 × 29 × 30 cm) and one larger chamber (30 × 29 × 30 cm) that contained a running wheel, food, and a water bottle. There were two passages between the chambers. Paternal behavior was assessed 2 days after the parents and pups were placed in the cage because paternal behavior is expressed at high levels during this time (Bester-Meredith, Young, and Marler, 1999). We removed the female and pups from the observation cage 90 s before the trial began. The pups were then returned to the chamber opposite their nest. The male was videotaped for 10 min with the pups, after which the female was returned to the cage. A single observer recorded the amount of time the males spent crouching over the pups (huddling), the amount of time the male spent licking the pups (pup grooming), and the amount of time the male spent retrieving pups.

The night after the paternal behavior test, we conducted a resident–intruder aggression test. Ninety seconds before the trial began, the female and pups were removed from the observation cage. Next, an unfamiliar, sexually inexperienced adult male was introduced into the chamber opposite the nest. We terminated a test after the resident attacked by biting the intruder, thereby preventing injury to the intruder. We measured attack latency, as this variable is negatively correlated with other measures of aggression in this species (Oyegbile and Marler, unpublished data). Individuals that are more aggressive are more likely to attack an intruder quickly (yielding a short attack latency). One test was omitted because the intruder attacked the resident (final $n = 30$). All data used for analyses were square root transformed, as Q-Q plots revealed that this transformation yielded the most normal distributions (Zar, 1996). Time spent huddling, grooming, and retrieving pups were regressed separately on attack latency.

### Castration Experiment: Males Observed with Foster Pups

To investigate the role of T in regulating paternal behavior and resident–intruder aggression, we performed a castration experiment using a different set of 30 males. Males underwent castration, castration + T replacement, or a sham procedure. Ten males were randomly assigned to each treatment group. We performed bilateral castrations through a single incision at the scrotum under isoflurane anesthesia. Both testes were removed using a sterile cautery, and the incision in the skin was closed using Nexaband adhesive. Ten castrated males were implanted subcutaneously with a 10-mm Silastic implant (i.d. 0.058, o.d. 0.077 in) packed with 1–2 mm of crystalline T. Actual T levels were confirmed with hormone assays (see below). We allowed each male 1 week to recover in isolation and 1 additional week while housed with its mate.

To assess behavior, males were placed in a Plexiglas observation cage (described above) 1 day prior to testing. This interval allowed subjects to become familiar with their surroundings, giving them an advantage for the resident–intruder aggression test. Soiled bedding from the pair's home cage was placed into the observation cage to provide olfactory stimulation for paternal behavior tests. Previous research has demonstrated that scents from a male's mate are important in the maintenance of paternal behavior (Gubernick and Alberts, 1989). On the second night, urine and feces from the female mate were obtained by lightly grasping the female on the back and holding her over a paper towel. A male subject was exposed to an unfamiliar 1- to 3-day old pup that had been wiped with urine and feces from its mate. The use of a single pup in this study is unlikely to bias the results, as litter size for California mice in the laboratory is typically only two or three pups, and an estimate of the average litter size in the field is less than two pups per litter (Gubernick and Teferi, 2000). Most males exhibited paternal behavior or ignored the pup. If the pup was attacked, then the test was immediately terminated. In the rare instances when the pup was injured, it was immediately euthanized. Males that ignored or provided care to the pup were videotaped for 10 min. Pups were used once for testing and then returned to their parents. On the third night, we measured attack latency in a resident–intruder aggression test as described above. All behavioral tests were scored by a single observer blind to treatment assignments. Initially, it was unclear how long the castration treatment would take to have an effect on behavior. Therefore, we tested all males for aggression and paternal behavior at 2, 3, and 4 weeks after treatment. A different 1- to 3-day-old pup and intruder were used each week for behavioral testing.

A blood sample was taken from the retro-orbital sinus 3 weeks after hormonal treatment (at least 2 days after the aggression test). All samples were collected in less than 2 min (mean ± SE 69 ± 4.8 s). Samples were immediately centrifuged and serum was stored at −80° C until assayed.

All behavioral variables were square root transformed, as Q-Q plots indicated that this transformation produced the most normally distributed data. Transformed data were analyzed using a repeated-measures ANOVA that tested for an effect of the hormonal treatment, week of testing, and treatment by week interaction. Cell variances were checked to verify the equality of variance assumption. We used Tukey posthoc comparisons to compare means.

### Castration Experiment: Males Observed with Their Own Pups

To control for any possible effects that foreign foster pups had on paternal behavior, we tested a different set of males with their own pups. Males were randomly assigned to undergo either a castration ($n = 6$) or sham ($n = 8$) procedure 3 days after the birth of a litter. This interval allowed for the males to fertilize their mates during the postpartum estrus, a period that lasts 1–3 days after birth (Gubernick, 1988). Males were given 1 week to recover in isolation, after which they were returned to their mates and pups. Pups were weaned at 30 days of age. Upon the birth of the next litter, the male, female, and pups were transferred to an observation cage. The female's left and right flanks were partially shaved for identification on videotapes. After a 3-day acclimation period, the male and female were videotaped for 30 min to observe male paternal behavior. Observation periods were scored by a single observer blind to treatment assignments. In addition to recording time spent huddling with and grooming pups, we also recorded time spent huddling with pups by the female and the amount of time the male spent inactive in the nest (i.e., sleeping). We reasoned that measuring inactivity in the nest would provide an estimate of passive association with pups, since a male sleeping in the nest would invariably be huddling with pups simultaneously. To investigate whether castration had any other effects on general activity or grooming behavior, we recorded time spent running in a wheel and self-grooming. All variables were square root transformed for analysis.

### Hormone Assays

Hormone assays were completed at the Wisconsin Regional Primate Research Center (WRPRC). Serum samples were extracted in ethyl ether. Steroid hormones were separated using celite chromatography using the System I technique (Abraham, Buster, Lucas, Corrales, and Teller, 1972). External recoveries of tritiated T (2500 cpm [$^3$H]T) were run in triplicate to estimate procedural loss. Sonification in an ultrasonic cleaner was used to keep extracted steroids suspended in 10% ethyl acetate in isooctane (EA / ISO) for application to columns. Testosterone was eluted with 4 ml of 20% EA / ISO. One sample from a T-replacement male and one sample from a castrated male were inadvertently lost during the chromatography procedure.

Testosterone was measured using an enzyme immunoassay (EIA) procedure modified by Ginther, Ziegler, and Snowdon (2001) from Munro and Stabenfeldt (1984). Briefly, microtiter plates (Nunc-Immuno Plate Maxisorb F96 certified, VWR Scientific, Chicago, IL) were coated with T antibody (R156, University of California–Davis diluted to 1:35,000) with coating buffer aliquoted in 100-$\mu$l amounts per well. Column-separated T fractions and standards (0.5–100 pg, $n = 8$, Sigma Diagnostics, Inc., St. Louis, MO) were assayed on microtiter plates. Absorbance was read at 420 nm on a Spectramax 340 (Molecular Devices Corp., Sunnyvale, CA). Data reductions (log-logit transformation) were analyzed by weighted least-squares regression analysis and reported as nanograms per milliliter of serum.

When assay concentrations for serial dilutions of a T-spiked California mouse serum pool (100–0.8 $\mu$l, $n = 8$) were compared with T standards, computed regression lines did not differ in slope ($t_{28} = 0.58$, $P > 0.5$). The sensitivity of the T EIA at 90% binding was 0.9 pg. Accuracy measured at each standard curve point (0.5–100 pg, 60 $\mu$l serum, $n = 8$) was 97.12 ± 1.61%. Quality control pools were assayed in duplicate at two different dilutions on each microtiter plate; the intra- and interassay coefficients for the 1:29 dilution were 3.2 and 10.1%, respectively, and for the 1:4 dilution were 2.1 and 7.6%, respectively ($n = 3$ plates). Nonparametric correlations were used to correlate hormone data with behavioral data because the sample size in each treatment group was relatively small.

## RESULTS

### Correlational Study

There was a negative correlation between huddling behavior and attack latency. Huddling behavior was significantly correlated with attack latency ($r =$

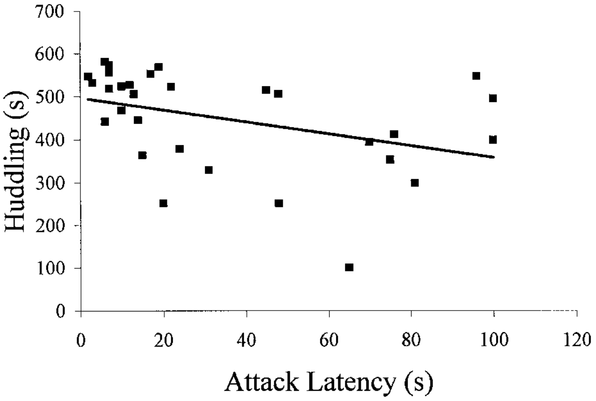

**FIG. 1.** Huddling behavior scores and attack latencies for each of the 30 males tested in the correlational experiment.

$-0.42$, $F(1, 28) = 6.18$ $P = 0.02$, Fig. 1). The relationship between pup grooming and attack latency was negative, but not significant. Males spent relatively little time retrieving pups during the 10-min tests (mean ± SE, 10.5 ± 3.7 s), and time spent retrieving pups was not significantly correlated with attack latency. Most males attended to the pups at the location where the pups were reintroduced to the cage.

### *Castration Experiment: Males Observed with Foster Pups*

Overall, sham and T-replacement males showed higher levels of paternal behavior than castrated males (Fig. 2). Repeated-measures analyses detected significant differences among treatments averaged across the 3 weeks of testing for huddling ($F(2, 27) = 12.7$, $P < 0.001$) and pup grooming ($F(2, 27) = 9.27$, $P < 0.001$) behavior but not pup-retrieving behavior. Pairwise Tukey comparisons indicated that castrated males showed significantly less huddling behavior than sham ($P < 0.05$) or T-replacement males ($P < 0.01$). Castrated males spent significantly less time grooming pups than T-replacement males ($P < 0.01$), but did not differ significantly from sham males. Sham and T-replacement males did not differ significantly in time spent huddling or grooming pups.

Treatment did not affect mean levels of aggression averaged across all 3 weeks of testing. There was no significant difference among treatment groups in attack latency. Attack latency tended to decrease across weeks of testing, an effect driven primarily by a consistent decrease in attack latency across weeks in sham males. There was a nonsignificant trend for an effect of week of testing averaged across the three treatment groups (Fig. 3; $F(2, 54) = 2.99$, $P = 0.058$). Although there was no significant week by treatment interaction, one-way repeated-measures analysis of each treatment group indicated that there was a strong effect of week of testing in sham-operated males ($F(2, 18) = 5.56$, $P = 0.01$) but not in castrated or T-replacement males.

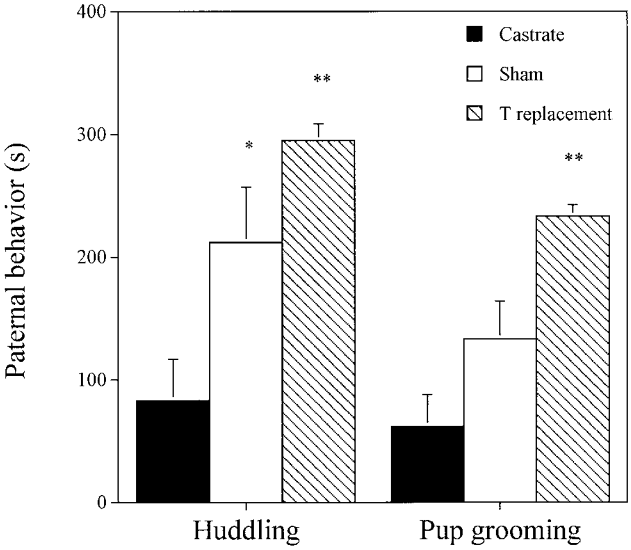

**FIG. 2.** Paternal behavior, averaged across weeks of testing for males tested with foster pups ($n = 10$ per group). Data are presented as means ± standard errors. Significantly different from castrates, $^*P < 0.05$, $^{**}P < 0.01$.

### *Castration Experiment: Males Observed with Their Own Pups*

As was observed in the previous experiment with foster pups, castrated males showed lower levels of

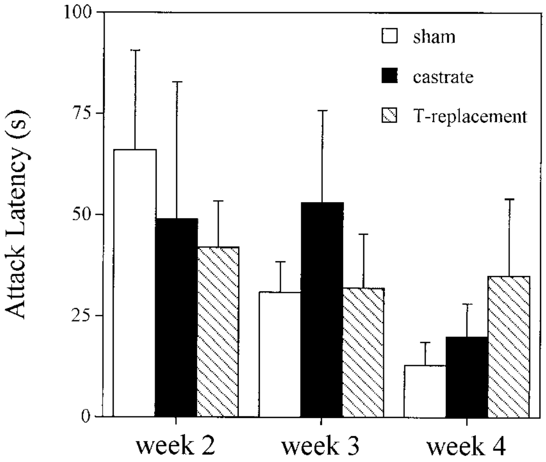

**FIG. 3.** Mean attack latencies plotted by week of testing ($n = 10$ per group). Data are presented as means ± standard errors.

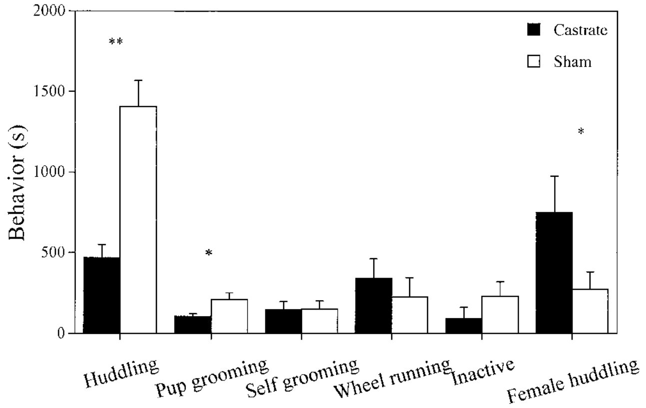

**FIG. 4.** Paternal and nonpaternal behavior from observations of castrated ($n = 6$) and sham males ($n = 8$) with their own pups. Data are presented as means ± standard errors. $**P < 0.001$, $*P < 0.05$.

paternal behavior than sham males. Castrated males spent significantly less time huddling with (Fig. 4; $t\,(12) = 4.80$, $P < 0.001$) and grooming ($t\,(12) = 2.20$, $P = 0.048$) their own pups than sham males. Pup retrievals were never observed in this experiment. There was no evidence that the differences in paternal behavior reflect changes in overall activity, as there were no differences in wheel running or self-grooming between sham and castrated males, and neither was there a difference in time spent inactive in the nest. Females paired with castrated males partially compensated for the decrease in paternal attention by increasing time spent huddling with pups compared to females paired with sham-operated males ($t\,(12) = 3.07$, $P = 0.01$).

### *Endocrine Data*

Sham males and T-replacement males had significantly higher T levels than castrated males, and T-replacement males had significantly higher T levels than sham males. One-way ANOVA revealed a significant effect of treatment on baseline T levels (Fig. 5; $F(2, 24) = 55.7$, $P < 0.001$). Pairwise Tukey comparisons indicated that castrated males had significantly lower T levels than sham ($P < 0.01$) and T-replacement males ($P < 0.001$), and T-replacement males had significantly higher levels of T than sham males ($P < 0.001$). Two castrated T-replacement males had T levels outside of the observed physiological range for California mice (greater than 1.2 ng / ml), but were well within the physiological range for other *Peromyscus* species (Trainor and Marler, unpublished data). These males did not show any overtly abnormal behavior.

There was a nonsignificant positive correlation between T and huddling behavior averaged across weeks of testing for sham-operated males (Spearman rank correlation, $\rho = 0.61$, $n = 10$, $P = 0.06$). Testosterone was not correlated with huddling behavior in castrated or T-replacement males. The correlations reflect a positive association between T and huddling behavior at intermediate levels of T that asymptote at higher levels of T. Testosterone was not consistently correlated with attack latency measured at any time point in castrated or T-replacement males. For sham males, there was a nonsignificant positive trend between T and attack latency at week 2 of testing. However, there was no significant correlation between attack latency at week 3 or week 4.

## DISCUSSION

We originally predicted that decreased T levels would increase levels of paternal behavior and decrease aggression. We based this prediction largely on the effects that T has on these behaviors in avian species and the eventual decrease in T seen in biparental gerbils (Brown *et al.*, 1995) and hamsters (Reburn and Wynne-Edwards, 1999) after parturition. In our study, however, we show convincing evidence that T maintains paternal behavior in male California mice. These results require a reconsideration of the role that T plays in mammalian paternal behavior. We

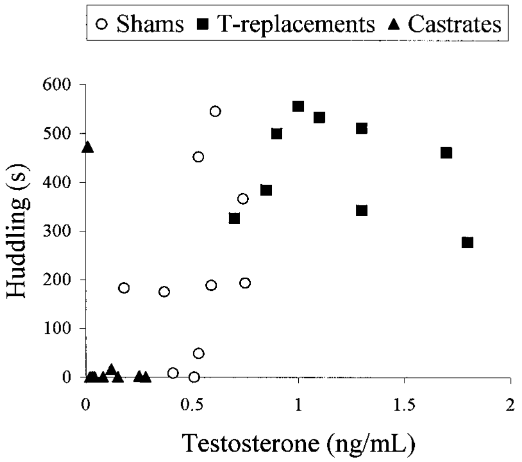

**FIG. 5.** Huddling behavior scores averaged across the three weeks of testing for castrates ($n = 10$), shams ($n = 9$), and T-replacement males ($n = 9$) plotted against T levels.

also show that baseline T levels do not maintain aggressive behavior, but that the ability for an individual to alter T levels may be important in repeated aggressive encounters. Finally, we show that aggression and paternal behavior are positively correlated.

### *Effects of T on Paternal Behavior*

The most striking observation is the reduction in paternal behavior in castrated males. Although the effect of T on paternal behavior is commonly thought to be negative, its effects have not been examined in many mammalian species that naturally show paternal behavior. While castration increases paternal behavior in the Mongolian gerbil (Clark and Galef, 1999), the effect of castration in socially monogamous prairie voles has been inconsistent. Published reports have found a decrease (Wang and DeVries, 1993) or no change in paternal behavior (Lonstein and DeVries, 1999) following castration. Our data represent a comprehensive examination of the effect of T on paternal behavior in a paternal mammal. We have shown a decrease in paternal behavior using two different experimental methods and show the effects of T on paternal behavior across a range of T levels. These results show that T can have a positive effect on paternal behavior.

There are at least two possible explanations for the reduction of paternal behavior observed in castrated males and the corresponding increase in T-replacement males. One explanation is that castrated males experience an inability to mate, which leads to a decrease in paternal behavior. A copulation-induced, time-related inhibition of infanticide has been identified for the CF-1 stock of domestic mice (Vom Saal and Howard, 1982) and male rats (Mennella and Moltz, 1988). While photoperiod cycles have been identified as the primary environmental cue linking mating to inhibition of infanticide, changes in T could be involved with this neural mechanism (Perrigo, Belvin, and vom Saal, 1992). However, the presence of such a neural mechanism in California mice seems unlikely, as previous research suggests that the most important experiential factor influencing paternal behavior in California mice was the birth of a male's own pups (Gubernick, Schneider, and Jeannotte, 1994). In addition, no such timing mechanism has been found in wild-type mice (Palanza and Parmigiani, 1991) or in California mice (see Gubernick *et al.*, 1994, for discussion). Finally, castrated male California mice have been observed to successfully copulate with females up to 4 weeks after treatment (B.C.T., personal observation).

A second explanation is that T or its androgenic metabolites directly stimulate the expression of paternal behavior via androgen receptors. Male plasma T increases 1 day prior to parturition in the dwarf hamster, *Phodopus campbelli*, and remains elevated on the day of birth (Reburn and Wynne-Edwards, 1999). In male cotton-top tamarins, T levels in reproductively experienced males increase during the first 5 days after birth (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000). Increased T levels could potentially stimulate paternal behavior. This hypothesis is further supported by the correlation between T and huddling behavior in sham-operated males and the heightened levels of huddling seen in T-replacement males. A related explanation is that aromatization of T to estradiol ($E_2$) in the brain plays an important role in the expression of paternal behavior. In female rodents, high levels of $E_2$ play an important role in the expression of maternal care (Bridges, 1996). In male rats, $E_2$ implants in the medial preoptic area (MPOA) stimulate parental behavior (Rosenblatt and Cues, 1998). The MPOA is a critical site for the maintenance of maternal behavior in rats (Numan, 1974; Numan, Corodimas, Numan, Factor, and Piers, 1988), and studies in domestic mice have found that aromatase enzymes (which convert T to $E_2$) are active in the hypothalamus and preoptic area (reviewed in Hutchison and Steimer, 1984). An aromatization hypothesis has been proposed for the positive effects of T on paternal behavior in the male Barbary dove, *Streptopelia risoria* (Steimer and Hutchison, 1981), but remains relatively unexplored in mammalian species in which paternal behavior is an important life history component.

An unexpected finding was that T manipulations in males resulted in changes in female behavior. Females paired to castrated males expressed a higher level of huddling behavior than females paired to sham males. Although other behaviors were not measured, this suggests that there may be plasticity in maternal behavior. A previous study found that females housed with a male mate nursed pups more than females housed alone (Cantoni and Brown, 1997). It is difficult to make direct comparisons, however, because in our study food was available *ad libitum* and in the Cantoni and Brown study, individuals had to run in a wheel to obtain food. However, follow-up investigations of the plasticity of maternal behavior in California mice may be useful.

### Effects of T on Aggression

The castration treatment shed light on the physiological basis for aggressive behavior in male California mice. Neither castration nor castration + T replacement altered mean levels of aggression. In golden hamsters (Vandenbergh, 1971), domestic mice (Leshner and Moyer, 1975), and rats (Albert *et al.*, 1986) castration in adults leads to a reduction in aggression, and baseline T replacement partially reverses this effect. In contrast, a castration experiment in monogamous prairie voles found a nonsignificant trend for castrated males to be more aggressive than sham-operated males in a neutral aggression test and no difference in aggression in a resident intruder test (Demas *et al.*, 1999). Behaviorally, California mice and prairie voles resemble many tropical bird species in which prolonged elevation of baseline T levels is not necessary for the expression of aggressive behavior (white-browed sparrow weaver, Wingfield, Hegner, and Lewis, 1992; spotted antbird, Hau, Wikelski, Soma, and Wingfield, 2000). One commonality between California mice and tropical avian species is prolonged territorial defense, even outside of the breeding season. Field studies have found that male and female California mice stay on their established territories until death and that pairs associate with one another throughout the year (Ribble, 1990; personal communication). This suggests that California mice defend territories throughout the year, as in many tropical avian species. The maintenance of year-round territories in tropical birds and California mice may favor the dissociation of male aggression and long-term elevation of baseline T levels. Prolonged elevation of T may have important costs such as increased mortality due to increased metabolic demands (Marler, Walsberg, White, and Moore, 1995) and the potential for immunosuppression (Hillgarth and Wingfield, 1997; Schuurs and Verheul, 1990; Zuk and McKean, 1996).

We found that only sham-operated males and not T-replacement males showed changes in aggressive behavior across weeks of testing. One possible explanation for these results is that short-term changes in T levels play an important role in the regulation of aggressive behavior over time. Studies in the neotropical spotted antbird show that T levels of territory residents increase after aggressive encounters (Wikelski, Hau, and Wingfield, 1999). Studies in rhesus monkeys (Rose, Holaday, and Bernstein, 1971) and humans (Suay, Salvador, González-Bono, Sanchís, Martínez, Martínez-Sanchis, Simón, and Montoro, 1999) have also found increases in T after aggressive encounters, suggesting that this may be a general response. Testosterone often increases in response to aggressive encounters, and only sham males could modulate T levels. Therefore we hypothesize that an increase in T after an aggressive encounter may increase aggression in males that win encounters. It is currently unknown whether transient changes in T make individuals more likely to win future encounters, although male California mice that win more encounters are more aggressive in future encounters (Oyegbile and Marler, unpublished data).

### The Relationship between Aggression and Paternal Behavior

Paternal behavior was negatively correlated with attack latency, indicating that males who were more paternal were also more aggressive. Unlike patterns seen in seasonally breeding birds, male California mice that spent more time huddling with their own pups tended to be more aggressive (have shorter attack latencies) in encounters with intruder males. There are several potential explanations for the differences seen between male California mice and seasonally breeding birds. Male California mice have baseline levels of T that do not appear to maintain aggressive behavior, whereas in many seasonally breeding avian species aggression is at least partially dependent on elevations of baseline T levels (Balthazart, 1983; Beletsky, Orians, and Wingfield, 1990). In addition, male California mice show high levels of aggression while expressing paternal behavior, whereas male aggression subsides in many avian species when paternal behavior is expressed (Wingfield and Farner, 1980; Ketterson, Nolan, Wolf, and Ziegenfus, 1992). This difference may be related to the postpartum estrus in California mice, as females are fertile soon after the birth of a litter (Gubernick, 1988). Aggressive mate-guarding by the male may be important, as laboratory studies have found that some paired females will mate with an unfamiliar male when her mate is tethered and unable to restrict mating activity (Gubernick and Nordby, 1993). This observation combined with the absence of extra-pair young in the field (Ribble, 1991) strongly suggests an important role for mate-guarding. Unfortunately, our data do not allow us to determine which, if any, of these aspects of the California mouse's ecology contribute to the observed patterns of behavior. Understanding which ecological parameters influence the relationship between aggression and paternal behavior will require studies of other biparental rodents with different ecologies.

In summary, we have demonstrated an important role for T in the maintenance of paternal behavior. These results suggest that T may have important positive effects on mammalian paternal behavior. In addition, the data in this and other studies suggest that in year-round territory holders, elevated baseline T levels are not required for the expression of aggressive behavior. The study of additional model species with prolonged territorial defense will be useful for determining whether the patterns of aggression and paternal behavior described in the present study can be generalized.

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