

Interactions among Paternal Behavior, Steroid Hormones, and Parental Experience in Male Marmosets (*Callithrix kuhlii*)

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Male black tufted-ear marmosets (*Callithrix kuhlii*) contribute to the rearing of their offspring. Here we evaluated predictions of hypotheses suggesting that (1) T and E₂ influence infant-care behavior in male marmosets, (2) levels of T and E₂ are modulated by paternal experience, and (3) paternal behavior and levels of T and E₂ in male marmosets covary with stress. We observed the behavior of marmosets in their family groups following the birth of infants and evaluated urinary concentrations of T, E₂, and the stress hormone cortisol (CORT) among fathers before and after the birth of young. Urinary levels of T, E₂, and CORT were lower among males who carried infants at high rates than males who carried at low rates, and T and CORT levels were negatively correlated with carrying rates across all males. Males had significantly lower T levels while carrying the second compared to the first litter and slightly lower rates of infant-carrying, possibly due to assistance provided by offspring of the first litter. There were increases in CORT levels of fathers after the birth of the first litter, but decreases in CORT after the birth of the second. Our results suggest a relationship in *C. kuhlii* between paternal behavior, hormones, and paternal experience. Rates of infant-carrying appear to be linked to hormone levels, and hormone levels in turn are affected by experience caring for young. Our data also suggest that T, E₂, and CORT have synergistic influences on infant-carrying behavior or alternatively that associations between T and E₂ and rates of infant-carrying are influenced by stress or other glucocorticoid-related variables. Finally, we propose a hypothesis suggesting that experience-related changes in hormones reinforce the commitment of males to successful breeding partnerships. © 2001 Academic Press

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In mammals, survival of young depends on assistance from parents, and mammalian species are characterized by extensive maternal investment of care in offspring (Trivers, 1972; Maynard Smith, 1977). In a small but diverse array of mammals, fathers also contribute to the rearing of offspring (Solomon and French, 1997). In these species the energetic demands of raising young are high, and natural selection has apparently favored fathers who help rear their offspring (Creel and Creel, 1991; Gubernick, Wright, and Brown, 1993; Emlen, 1995).

Among mammalian species in which fathers contribute to the care of young, distinct relationships exist between hormones and parental behavior. Males in a variety of mammalian species experience elevations in prolactin (PRL) during periods in which they care for young, suggesting that PRL commonly promotes mammalian paternal behavior (Dixson and George, 1982; Gubernick and Nelson, 1989; Brown, Murdoch, Murphy, and Moger, 1995; Ziegler, Wegner, and Snowdon, 1996; Ziegler and Snowdon, 1997; Reburn and Wynne-Edwards, 1999). Steroid hormones also influence mammalian paternal behavior; however, their effects are less clear and consistent than those of PRL. Expression of paternal behavior is independent of testosterone (T) in some mammalian species, including rats, common marmosets, and California mice (Svare, Bartke, and Gandelman, 1977; Dixson and George, 1982; Gubernick and Nelson, 1989). However, in prairie voles, T appears to promote care of young in fathers (Wang and DeVries, 1993), whereas in Mongolian gerbils, biparental dwarf hamsters, humans, and black tufted-ear marmosets T appears to hinder paternal care, possibly by mediating behaviors such as courtship or aggression which potentially interfere

with nurturing young (Brown *et al.*, 1995; Clark, De-sousa, Vonk, and Galef, 1997; Reburn and Wynne-Edwards, 1999; Storey, Walsh, Quinton, and Wynne-Edwards, 2000; Nunes, Fite, and French, 2000). The role of estrogens in mediating mammalian paternal behavior has not been widely investigated. However, preliminary evidence suggests that, as is the case with T, estrogens may promote paternal care of young in some species (e.g., rats: Rosenblatt, Wagner, and Morrell, 1994; Rosenblatt and Ceus, 1998), but antagonize the expression of paternal behavior in others (e.g., black tufted-ear marmosets: Nunes *et al.*, 2000).

The proficiency of parental and other reproductive behaviors is often enhanced by experience (e.g., Epple, 1978; Holekamp and Talamantes, 1992; Pryce, 1993; Wang and Novak, 1994; Fleming, Morgan, and Walsh, 1996). In male lab rats and mice, for example, paternal responsiveness increases with the duration of exposure to pups (Mayer, Freeman, and Rosenblatt, 1979; Brown, 1985). Parenting experience also influences hormone levels in a variety of mammalian species. For example, male cotton-top tamarins with prior experience raising young tend to have higher PRL and lower cortisol (CORT) levels than first time fathers (Ziegler *et al.*, 1996), and male black tufted-ear marmosets with a great deal of prior experience caring for infants tend to have lower T and estradiol (E_2) levels than males with little or no experience (Nunes *et al.*, 2000). The influence of parental experience on both behavior and hormone levels in mammals raises the possibility that experience affects some elements of parental behavior via modulation of hormone levels. We note, however, that some components of parental behavior become increasingly independent of hormones as parents gain experience caring for young (Fleming *et al.*, 1996).

Our objective here was to examine the relationship between parental behavior, steroid hormones, and experience raising offspring in male black tufted-ear marmosets (*Callithrix kuhlii*). In prior work we observed that urinary concentrations of T and E_2 in male *C. kuhlii* decline after young are born, reaching their lowest levels during the period in which fathers carry infants at peak rates (Nunes *et al.*, 2000). In the current study we examined whether variation in the amount of care individual males invest in young is related to their hormone levels. We also examined whether males' hormone levels are influenced by prior paternal experience, as suggested by Nunes *et al.* (2000). Experience-related variation in hormones might reflect changes in responses to stress, so we also examined the possibility that sex steroid hormone levels of fathers covary with the stress hormone CORT (Smith

and French, 1997). We observed the behavior of marmosets in their family groups and measured urinary T, E_2 , and CORT concentrations of adult males during the rearing of two consecutive litters. First, we predicted that if T and E_2 influence infant care in male marmosets, then levels should differ between males that engage in extensive paternal care and those that engage in lower amounts of care, and levels of these hormones should be correlated with rates of infant-carrying behavior. Second, we predicted that if T and E_2 levels are modulated by paternal experience, then urinary T and E_2 concentrations should change predictably between consecutive litters. Finally, we predicted that if stress influences the relationship between paternal behavior, T and E_2 , and experience, then CORT levels of males should be correlated with these variables.

METHODS

Subject Animals

C. kuhlii are small, tree-dwelling primates native to forests near the Atlantic coast of South America (Nowak, 1991). Males reach reproductive maturity at ca. 12 months of age (French and Schffner, 1995), and females at 12–15 months (Smith *et al.*, 1997). *C. kuhlii* appear to follow the typical callitrichid pattern of residing in family groups and not mating until well after puberty, when a vacancy in the breeding pair arises in their own or a neighboring group. Gestation in *C. kuhlii* lasts ca 4.5 months (French *et al.*, 1996). Female *C. kuhlii* experience postpartum estrus 5–12 days after giving birth and thus have the potential to bear successive litters ca. 5 months apart (French *et al.*, 1996). Females usually give birth to twins, and litters comprise up to 25% of a mother's body mass (Leutenegger, 1973; Santos *et al.*, 1997). Fathers and siblings contribute to the care of infants, primarily by carrying and warming them, sharing solid food with them after weaning, and protecting them from danger (Santos *et al.*, 1997).

From August 1996 to September 1999 we studied the behavioral endocrinology of nine adult male *C. kuhlii* housed at the Callitrichid Research Center at the University of Nebraska at Omaha. Animals were kept in large, indoor enclosures ($1.6 \times 0.9 \times 2.4$ or $1.0 \times 0.9 \times 2.4$ m), each equipped with a plastic sleeping tube and platforms and natural branches for climbing. Large sheets of opaque corrugated plastic restricted visibility between enclosures in the same room of the

TABLE 1

Carrying Behavior, Age, and Parenting Experience of Individual Male *C. kuhlii* and Composition of Their Family Groups

ID	First litter included in study					Second litter included in study				
	Mean carrying rate (min/h)	Age (months)	No. of prior litters fathered	Time since previous litter (months)	No. of older offspring in group	Mean carrying rate (min/h)	Age (months)	No. of prior litters fathered	Time since previous litter (months)	No. of older offspring in group
Ren	40.8	68	0	—	0	—	—	—	—	—
Fig	32.3	28	0	—	1 ^a	29.7	33	1	5	3
Hum	32.2	148	4	30	1	27.0	153	5	5	3
Den	28.0	86	1	35	1	—	—	—	—	—
Moz	24.9	74	0	—	0	N/A	79	1	5	2
Kip	22.5	38	0	—	0	12.7	43	1	5	2
Geo	20.2	67	8	11	3	—	—	—	—	—
Gro	11.6	43	0	—	0	5.2	48	1	5	2
Hea	4.0	71	1	17	1	13.8	76	2	5	3

^a Stepdaughter.

research center. Animals were fed once daily at ca. 0800 h, and water was provided *ad libitum*. Schaffner *et al.* (1995) provide specific details of animal care and husbandry procedures in this colony.

Experimental Design

Our first objective was to evaluate the infant-carrying behavior of adult male *C. kuhlii* relative to that of other members of their family groups and to compare urinary steroid hormone concentrations between males who carried infants at high hourly rates and those who carried young at low rates. To do this, we observed the behavior of marmosets in nine different family groups after the birth of twin infants and measured urinary T, E₂, and CORT levels in urine samples collected from adult males prior to and after the birth of their offspring (see below). We later evaluated the infant-carrying behavior of group members and the hormone levels of fathers during 2-week time intervals. We compared behavior among time intervals and group members using a mixed within-/between-subjects design, with time as a repeated, within-subjects variable and category of group member as a between-subjects variable. We compared hormone levels among time intervals and between fathers who carried young at high vs low hourly rates, also using a mixed within-/between-subjects design, with time as a within-subjects variable and carrying effort as a between-subjects variable. Since the distribution of carrying effort among males was continuous, we performed a median split among males as a function of their carrying effort, and four males were identified as

High Effort carriers, and five males were identified as Low Effort carriers. We also used correlation analysis to assess the relationship between infant-carrying behavior and hormone levels among all nine males. Family groups were considered independent sampling units. The nine adult males included in this phase of our work are described in Table 1.

Our second objective was to elucidate the effect of experience raising litters on the behavior and urinary steroid hormone concentrations of adult male *C. kuhlii*. To do this, we compared the behavior of marmosets in family groups, and hormone levels of fathers, between the rearing of two consecutive twin litters born 5 months apart. Our analysis of behavior used a mixed design, with time and rearing of litters as repeated, within-subjects variables and category of group members as a between-subjects variable. Our analysis of fathers' hormone levels used a completely within-subjects design, with time and rearing of litters as repeated variables. Family groups were considered independent sampling units. Descriptions of the six adult males used in this phase of our work are presented in Table 1.

Observations of Behavior

We observed the behavior of marmosets for a total of 167 h using the focal animal survey technique of Altmann (1974). We observed each family group for 8 weeks following the birth of infants. Behavioral surveys lasted 20 min, and were conducted on average 4.5 ± 0.6 (SD) times per week for each group at randomly selected times between 0900 and 1700 h. Dur-

ing surveys, an observer familiar to the focal group sat approximately 2 m from its enclosure and recorded the behavior of group members using Observer 3.0 software programmed onto a 486 laptop computer. We later calculated hourly rates of infant-carrying during 2-week time intervals. We defined infant-carrying behavior of marmosets as conducting typical activities with one or more infants clinging to the fur or body. Behavioral data from the rearing of one father's (Moz, see Table 1) second litter were incomplete and excluded from the study.

Urine Collection

We collected one to three urine samples per week from adult males, beginning 5 weeks before and continuing until 8 weeks after the birth of young, using noninvasive techniques described by French *et al.* (1996). Briefly, we trained subjects to urinate into small, hand-held pans in return for food rewards. We collected 0.2–1.0 ml of urine from each subject between 0600 and 0800 h, immediately after the animals first woke up in the morning. We transferred urine samples to plastic vials, centrifuged them at 700 rpm for 2 min to separate urine from sediments, then transferred the supernatant portion of each sample to a clean vial, and stored it at -20°C until it was assayed for hormone concentrations.

Hormone Assays

Concentrations of T, E_2 , and CORT in urine samples were determined using enzyme immunoassays described and validated for use with *C. kuhlii* in prior work (T, E_2 : Nunes *et al.*, 2000; CORT: Smith and French, 1997). We extracted T and E_2 from samples with diethyl ether before performing assays (Nunes *et al.*, 2000). Mean recovery of hormones during extractions was 95.0% for T and 84.9% for E_2 . We measured hormone concentrations in 12 T assays, 14 E_2 assays, and 12 CORT assays and adjusted T and E_2 levels for procedural losses during extractions. Intra-assay coefficients of variation, determined from duplicate evaluations of pooled marmoset urine run within assays, were 4.0, 3.4, and 1.7% for high concentration pools and 2.9, 4.6, and 2.1% for low concentration pools in T, E_2 , and CORT assays, respectively. Interassay coefficients of variation, also determined from evaluations of pooled marmoset urine run within assays, were 10.0, 19.9, and 7.8% for high concentration pools and 18.3, 14.6, and 20.1% for low concentration pools in T, E_2 , and CORT assays, respectively. To minimize the

potential confounding effects of interassay variation, all samples collected from an individual male were evaluated in a single assay when possible. When this was not possible, all samples collected from an individual during the rearing of a litter were run in a single assay. Because most interassay variation occurred between assays run on different days, all samples collected from an individual male were run on the same day, and males were assigned randomly to days for assays.

To control for variation in solute concentration of urine samples, we expressed the mass of T, E_2 , and CORT per milligram of creatinine (Cr), a product of muscle metabolism excreted at consistent rates. We measured Cr in urine samples using a modified Jaffe end-point assay (Tietz, 1976), previously described and validated for *C. kuhlii* (French *et al.*, 1996).

Statistics

Dependent measures monitored in our study included hourly rates of behavior, urinary concentrations of T, E_2 , and CORT, and levels of T, E_2 , and CORT expressed as a proportion of prepartum concentrations. These data were normally distributed (Liliefors test, $P > 0.05$) and evaluated with repeated measures ANOVA or mixed-design ANOVA. When ANOVA indicated significant overall differences, we used paired or independent *t* tests to compare means between specific time intervals or groups, adjusting significance levels of multiple pairwise comparisons with Bonferroni's procedure. We used Pearson's correlations to evaluate the association between rates of infant-carrying behavior and hormone levels. Mean values are presented ± 1 SEM unless otherwise indicated. We defined observed differences to be significant when $P \leq 0.05$.

RESULTS

Postpartum Variation in Infant-Carrying and Hormones

Rates of infant-carrying behavior, and the patterns of change in infant-carrying as the infants matured, differed among members of *C. kuhlii* family groups. We evaluated rates at which fathers, mothers, and siblings carried at least one infant from a twin litter during the 8 weeks following parturition and observed significant variation overall among these caregivers (Fig. 1; ANOVA, $F(2, 20) = 4.436$, $P = 0.025$).

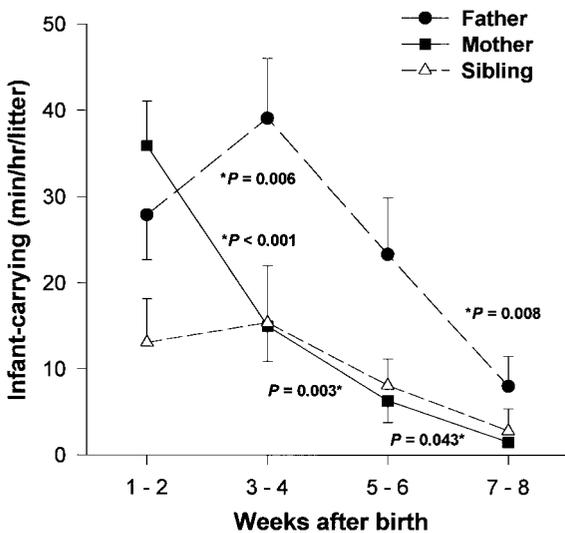


FIG. 1. Rates at which infant *C. kuhlii* were carried by various group members. Asterisks indicate significant differences between successive time intervals, determined by paired *t* tests. Sample included nine twin litters.

We also observed significant variation in carrying behavior over time (Fig. 1; ANOVA, $F(3, 60) = 17.275$, $P < 0.001$) and an interaction between time and identity of carrier (Fig. 1; ANOVA, $F(6, 60) = 4.419$, $P = 0.001$), indicating that temporal patterns of change in infant carrying differed between fathers, mothers, and siblings. Mothers carried young at peak rates 1–2 weeks after parturition, whereas peak carrying behav-

ior of fathers occurred later, 3–4 weeks after birth. Rates at which infants were carried by their siblings were low overall, peaking 1–4 weeks after parturition. The mean overall rate of infant-carrying during the 8 week postpartum period was significantly greater among fathers than either mothers (paired *t* test, $n = 9$, $t_8 = 2.694$, $P = 0.027$) or siblings (paired *t* test, $n = 5$, $t_4 = 2.832$, $P = 0.047$). We also observed a trend that approached significance for overall rates of infant-carrying to be greater among mothers than siblings (paired *t* test, $n = 5$, $t_4 = 2.673$, $P = 0.056$). Thus, mothers and fathers bore the primary responsibility of carrying infants, with both parents carrying at high rates during the first 2 weeks after birth and fathers serving as the principal carriers thereafter.

We evaluated the association between carrying effort and urinary concentrations of T, E₂, and CORT of fathers during the 8 weeks following the birth of young by contrasting hormone levels between fathers that carried infants at high vs low rates and by correlating postpartum hormone concentrations with hourly rates of infant-carrying behavior. First, we split our sample of males into High Effort or Low Effort carriers, based on their average hourly rates of infant-carrying behavior. Males in these two groups did not differ significantly with respect to age (Fig. 2A; independent *t* test, $t_7 = 0.916$, $P = 0.417$), the number of prior litters they raised as a father (Fig. 2B; $t_7 = 0.281$, $P = 0.787$), or the number of older offspring with whom they resided (Fig. 2C; $t_7 = 0.072$, $P = 0.945$). As

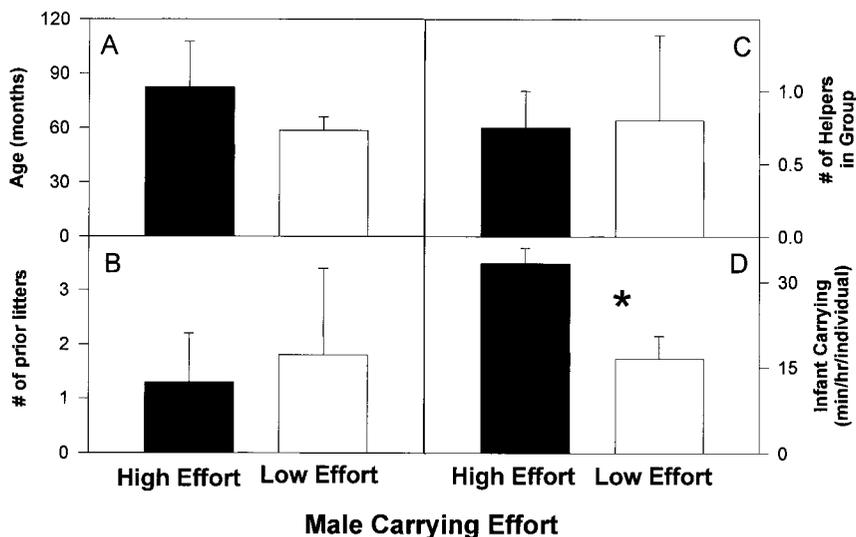


FIG. 2. Comparisons of (A) age, (B) paternal experience, (C) home group size, and (D) infant-carrying between male *C. kuhlii* who carried infants at high vs low rates. The asterisk indicates a significant difference ($P < 0.05$) between groups of males.

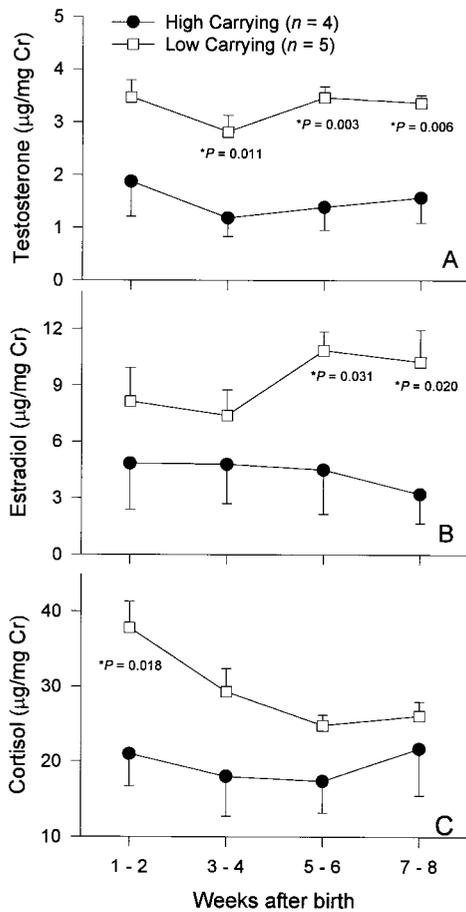


FIG. 3. Urinary concentrations of (A) testosterone, (B) estradiol, and (C) cortisol of male *C. kuhlii* following the birth of young. Asterisks indicate significant differences between males who carried infants at the highest vs lowest rates, determined by posthoc comparisons of means.

expected, however, the two classes of males differed significantly with respect to hourly rates at which they carried young (Fig. 2D; $t_7 = 3.345$, $P = 0.012$).

Urinary T concentrations were significantly greater among males who carried young at low rates compared to males who carried young at high rates (Fig. 3A; ANOVA, $F(1, 7) = 15.423$, $P = 0.006$) and varied significantly over time, declining to trough levels 3–4 weeks after the birth of young (Fig. 3A; ANOVA, $F(3, 21) = 3.178$, $P = 0.045$). Urinary E_2 and CORT concentrations tended to be greater among males who carried infants at low compared to high rates, but these differences only approached significance (Figs. 3B and 3C; E_2 , ANOVA, $F(1, 7) = 5.270$, $P = 0.055$; CORT, ANOVA, $F(1, 7) = 4.609$, $P = 0.069$). We also observed significant variation in CORT over time (Fig. 3C; ANOVA, $F(3, 21) = 5.270$, $P = 0.008$) and an interac-

tion between time and carrying effort that approached significance in our analysis of CORT (Fig. 3C; ANOVA, $F(3, 21) = 2.965$, $P = 0.055$). In particular, males who carried young at low rates had higher CORT levels 1–2 weeks after parturition than males who carried young at high rates; however, their CORT declined thereafter to levels comparable to those of males who carried infants at high rates. Overall, our results here indicate a distinct association in male *C. kuhlii* between T, E_2 , and CORT and infant-carrying behavior, with fathers who exhibit the highest rates of infant-carrying having the lowest hormone levels.

Among all nine males, correlational analyses revealed a similar relationship between hormone concentrations and carrying effort. Specifically, we observed significant negative correlations between infant-carrying and T and CORT levels of individual males during the first 6 weeks postpartum, when males are most actively engaged in infant care (T, $r = -0.70$, $P = 0.034$; CORT, $r = -0.77$, $P = 0.015$). There was also a negative relationship between infant-carrying and E_2 levels, but the correlation was not significant (E_2 , $r = -0.473$, $P = 0.19$).

We also observed a significant covariation among hormone levels in males. In particular, we observed significant positive correlations between urinary concentrations of T and E_2 (Fig. 4A; $n = 9$, $r = 0.906$, $P = 0.001$), T and CORT (Fig. 4B; $r = 0.978$, $P < 0.001$), and E_2 and CORT (Fig. 4C; $r = 0.857$, $P = 0.003$). Thus, males that had high T concentrations during the postpartum period also had high concentrations of urinary CORT and E_2 .

Changes in Infant-Carrying and Hormones between Consecutive Litters

We evaluated rates at which members of *C. kuhlii* family groups carried at least one infant during the rearing of consecutive twin litters that were born 5 months apart. Rates of infant-carrying behavior and patterns of change in these rates were similar to those observed in the first part of our study (Fig. 1 vs Fig. 5). Specifically, we observed a difference in rates of infant-carrying among fathers, mothers, and siblings which approached significance ($F(2, 12) = 3.634$, $P = 0.058$), significant variation in carrying behavior over time ($F(3, 36) = 21.912$, $P < 0.001$), and a significant interaction between time and category of family member ($F(6, 36) = 5.470$, $P < 0.001$).

There was a trend for the infant-carrying behavior of *C. kuhlii* to vary to a small degree between successive litters; however, this variation was not significant.

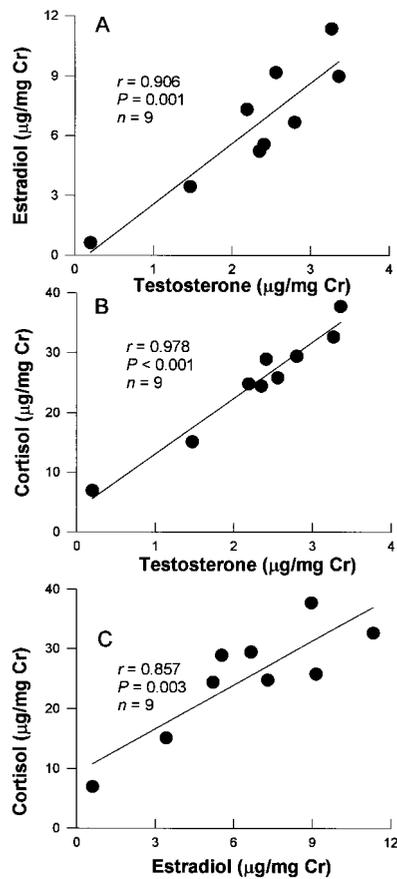


FIG. 4. Correlations in individual adult male *C. kuhlii* between urinary concentrations of: (A) testosterone and estradiol, (B) testosterone and cortisol, and (C) estradiol and cortisol.

In particular, parents carried infants at slightly lower rates, and siblings at slightly higher rates, while rearing the second compared to the first of consecutive litters (Fig. 5). We note here that litter composition differed between successive litters in our sample, with second litters each being composed of two more siblings than first litters (Table 1). This may account for some of the interlitter variation in infant-carrying behavior that we observed. Rates of infant-carrying by fathers during the first of successive litters were significantly correlated with carrying rates across 2-week intervals during the second of successive litters ($n = 20$, $r = 0.765$, $P < 0.001$). Thus, individual males appear to be consistent from litter to litter with respect to the amount of effort they allocate to carrying infants.

We also examined urinary concentrations of T, E_2 , and CORT among male *C. kuhlii* during the rearing of consecutive litters to assess the influence of raising

young on males' hormone levels. Urinary T concentrations of males declined between consecutive litters (Fig. 6A, $F(1, 5) = 14.451$, $P = 0.013$). Interlitter differences in T levels of males in our sample were most pronounced 1–4 weeks postpartum (Fig. 6A), when rates of infant-carrying behavior were greatest among these males (Fig. 5A). Thus, our results suggest a possible effect of prior infant-rearing experience on T levels of males while they are carrying young. We observed no significant differences in males' urinary E_2 or CORT concentrations between consecutive litters and no interactions between time and litter in our analysis of either T, E_2 , or CORT. Thus, absolute urinary concentrations of T, but not E_2 or CORT, during the period of infant care appear to decline as fathers gain experience rearing litters.

We also observed temporal variation in urinary hormone concentrations of males after their young were born. This variation approached significance in our

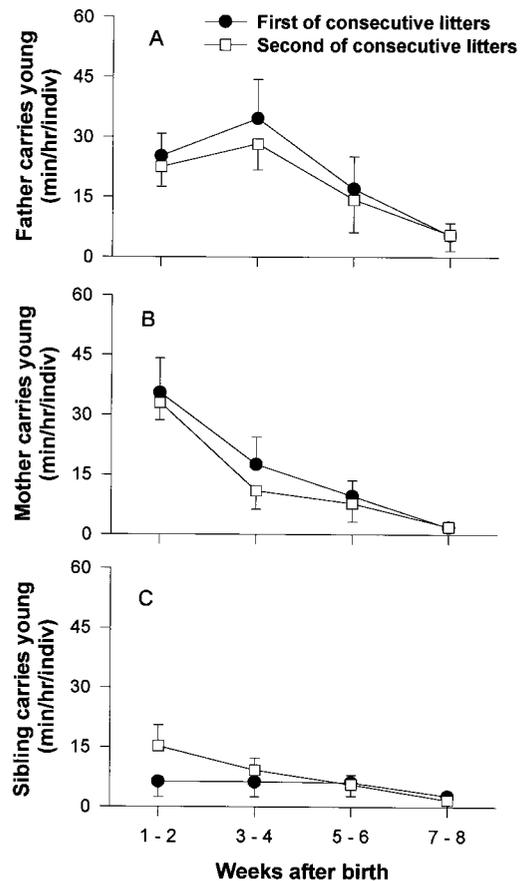


FIG. 5. Changes between consecutive *C. kuhlii* litters in infant-carrying by (A) fathers, (B) mothers, and (C) siblings. Sample included five pairs of twin litters.

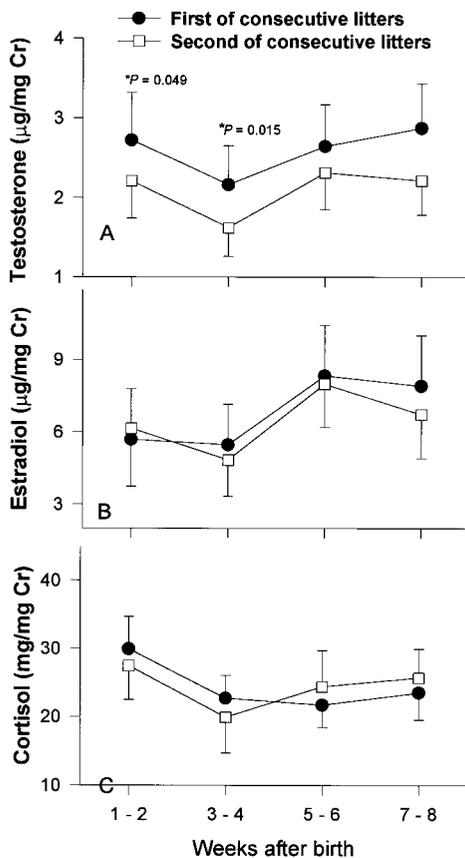


FIG. 6. Endocrine changes during the rearing of consecutive litters among male *C. kuhlii*. [(A) testosterone, (B) estradiol, and (C) cortisol]. Asterisks indicate significant differences between successive litters within time intervals, determined by paired *t* tests. Samples included six pairs of twin litters.

analysis of T and E_2 (Figs. 6A and 6B; T, $F(3, 15) = 2.669$, $P = 0.085$; E_2 , $F(3, 15) = 2.909$, $P = 0.069$) and was significant in our analysis of CORT (Fig. 6C; $F(3, 15) = 5.499$, $P = 0.009$). In particular, T levels of males were at their lowest 3–4 weeks after the birth of young, whereas E_2 levels were lowest 1–4 weeks postpartum, and CORT levels decreased 3–4 weeks after young were born and remained low thereafter (Fig. 6).

In prior work, we also observed postpartum periods during which levels of urinary T and E_2 in male *C. kuhlii* were relatively low. These low hormone levels reflected significant declines *within males* from prepartum concentrations (Nunes *et al.*, 2000). To assess whether infant-rearing experience influences the degree to which hormone concentrations decline after young are born, we evaluated urinary hormone levels of males expressed as a proportion of their prepartum concentrations. Proportional T levels of males differed significantly between consecutive litters (Fig. 7A, $F(1,$

5) = 9.882, $P = 0.026$). In particular, the degree of postpartum decline in T was greater following the birth of the second than the first of successive litters (Fig. 7A). The difference in degree of postpartum decline was most pronounced 3–4 weeks after birth (Fig. 7A), when males carried infants at peak rates (Fig. 5A). We observed no significant differences between the rearing of successive litters in E_2 or CORT levels of males expressed as a proportion of prepartum concentration. However, there was a significant interaction between time and litter in our analysis of CORT (Fig. 7C, $F(3, 15) = 3.291$, $P = 0.050$). In particular, CORT levels of males tended to increase immediately after the birth of the first of successive litters, but tended to decrease after the birth of the second. Thus, as male *C. kuhlii* gained experience in raising litters, the degree to which their T levels declined after the birth of young

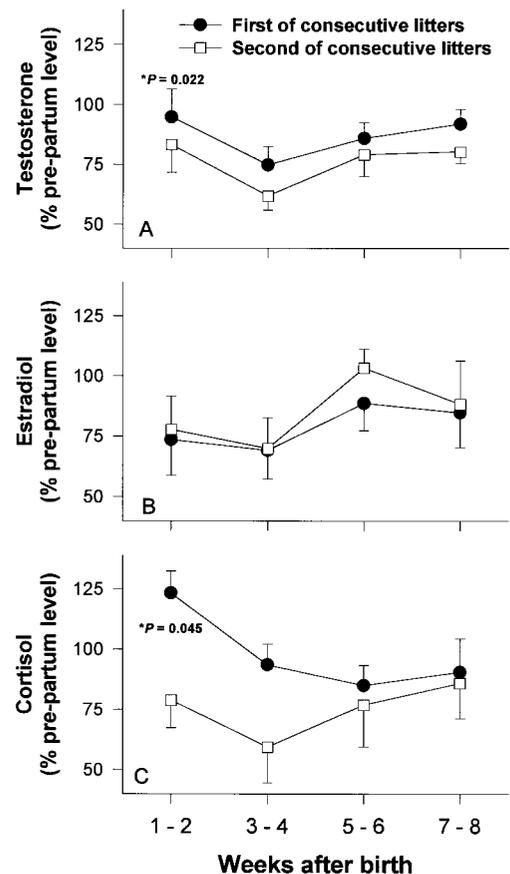


FIG. 7. Changes between the rearing of consecutive litters among male *C. kuhlii* in urinary levels of (A) testosterone, (B) estradiol, and (C) cortisol, expressed as a proportion of prepartum concentrations. Asterisks indicate significant differences between successive litters within time intervals, determined by paired *t* tests. Samples included six pairs of twin litters.

increased, and their CORT levels decreased rather than increased immediately after young were born.

DISCUSSION

Hormones and Infant-Carrying Behavior

Expression of paternal behavior in male *C. kuhlii* was associated with steroid hormone levels. Males who carried infants at high rates had lower absolute concentrations of urinary T and E_2 than did fathers who carried young at low rates, and there was a significant negative correlation between urinary T concentrations and hourly rates of infant-carrying behavior. These data are consistent with prior work indicating that T levels decline during periods of paternal care in *C. kuhlii* and suggest that T has antagonistic effects on paternal behavior in this species (Nunes et al., 2000), a phenomenon which has been observed in other mammals (Brown et al., 1995; Clark et al., 1997; Reburn and Wynne-Edwards, 1999; Storey et al., 2000), as well as several bird species (Silverin, 1980; Hegner and Wingfield, 1987; Wingfield, Hegner, Dufty, and Ball, 1990; Ketterson, Nolan, Wolf, and Ziegenfus, 1992; Oring, Fivizzani, and El Halawani, 1992; Mauget, Garcia, and Jouventin, 1995; Saino and Moller, 1995; Vleck and Brown, 1999). These data also raise the possibility that the effects of E_2 on paternal behavior in *C. kuhlii* are similar to those of T or that T and E_2 have synergistic effects on paternal behavior. Variation in both circulating and urinary T and E_2 levels in fact arise from testicular activity in male *C. kuhlii*, further supporting a role for E_2 in their reproductive physiology (Nunes, Brown, and French, submitted for publication).

Urinary CORT concentrations were also lower among male *C. kuhlii* who carried offspring at high compared to low rates and were negatively correlated with infant-carrying behavior among all males. Levels of CORT increase with stress-induced activation of the hypothalamus–pituitary–adrenal (HPA) axis in *C. kuhlii* and other mammals (Munk, Gyure, and Holbrook, 1984; Sapolsky and Meany, 1986; Smith and French, 1997). At a minimum, our data suggest that infant-carrying does not evoke a notable stress response in male *C. kuhlii*. Another possible interpretation of our data is that stress influences infant-carrying behavior as well as CORT levels in male *C. kuhlii*, causing stressed fathers to have high levels of CORT and carry infants at lower rates than fathers who are relatively unstressed. Increases in CORT are associ-

ated with variation in parental behavior and responsiveness to infants in some female primates (e.g., Fleming, Steiner, and Corter, 1997; Bahr, Pryce, Döbeli, and Martin, 1998). We note that seven of the nine males in this part of our study were novice fathers or had weaned only one litter in the past (Table 1), and lack of familiarity with raising young may have caused some of these fathers to become stressed by the birth of infants. Another possibility is that the contact with young that fathers experience during infant-carrying dampens their physiological stress responses, causing males who carry young at high rates to have low CORT levels. The presence of familiar conspecifics does in fact sometimes buffer stress responses in *C. kuhlii* and other mammals (e.g., Smith, McCreer-Whitworth, and French, 1998; Hennessy, 2000).

Stress-related activity along the HPA axis can suppress activity along the hypothalamus–pituitary–gonad (HPG) axis, resulting in decreased secretion of gonadal hormones (e.g., Sapolsky, 1985; Elman and Brier, 1997). However, in the current study we observed a positive rather than a negative correlation between levels of CORT and levels of T and E_2 in male *C. kuhlii*, suggesting that HPA activity does not suppress HPG activity during the period in which males care for infants. In some circumstances, activity along the HPA axis can stimulate HPG activity, resulting in increased secretion of gonadal hormones (e.g., Sapolsky, 1986; Siegel, Weidenfeld, Feldman, Conforti, and Chowers, 1991; Sapolsky, Romero, and Munck, 2000). The positive correlations we observed between T and E_2 levels of male *C. kuhlii* and their CORT levels thus raise the potential that HPG effects upon paternal behavior are influenced in some capacity by HPA activity.

In prior work we observed that postpartum declines in T levels of male *C. kuhlii* do not require continued stimuli from infants after they are born. However, the possibility remains that endocrine variation in paternal *C. kuhlii* may be triggered by chemosignals that males receive from their breeding partners or offspring before or near the time of parturition (Nunes et al., 2000). Some male mammals can in fact detect cues from females that vary with females' reproductive state and hormone levels (e.g., Ziegler, Epple, Snowden, Porter, Belcher, and Kuderling, 1993). Furthermore, a growing body of data suggests that cues from female mammals can induce paternal changes in hormone levels of males. For example, changes in PRL experienced by paternal cotton-top tamarins appear to be signaled by cues from their pairmates prior to the birth of young (Ziegler et al., 1996). Moreover, in hu-

mans, pre- and postpartum hormonal changes follow similar patterns in mothers and fathers, suggesting that cues from women may trigger hormonal variation in paternal men (Storey *et al.*, 2000).

Although our data presented here and elsewhere (Nunes *et al.*, 2000) provide converging evidence for a causal link between variation in hormone levels and variation in paternal care in male *C. kuhlii*, our research designs to this point have been characterized by a correlational strategy. Experimental manipulation of hormone levels in future work is needed to fully establish a causal relationship between hormones and paternal behavior. For example, if T is in fact antagonistic to paternal care of young in *C. kuhlii*, then experimentally increasing or decreasing T levels of males should cause inverse changes in their infant-carrying behavior. Moreover, experimentally manipulating CORT levels of males and observing the effects on T and E_2 levels, and subsequent changes in infant-carrying behavior would help to elucidate the relationship among HPG and HPA regulation of male parental responsiveness.

Effects of Experience on Hormones and Infant-Carrying

Paternal experience affected the dynamics of T excretion in male *C. kuhlii*. Urinary T concentrations were significantly lower among males while rearing the second compared to the first of consecutive litters, suggesting that T levels of males decline as they acquire experience raising infants. Moreover, urinary T levels of males expressed as a proportion of prepartum concentrations were lower while rearing the second compared to the first of successive litters, suggesting that paternal experience also influences the magnitude of declines in T that ordinarily accompany the onset of paternal care in *C. kuhlii* (Nunes *et al.*, 2000). We note, however, that the effects of experience on T levels of male *C. kuhlii* were not observed for E_2 , suggesting that experience raising young differentially influences T and E_2 levels of males. Endocrine changes associated with the acquisition of paternal experience have similarly been observed in cotton-top tamarins (Ziegler *et al.*, 1996). Tamarin fathers with prior experience raising offspring tend to have higher PRL levels than first-time fathers. However, some of the variation in PRL observed by Ziegler *et al.* (1996) may have been due to changes associated with aging, since experienced fathers were markedly older than inexperienced males, and PRL titers decrease with age in a variety of male mammals (Meites *et al.*, 1980; Goya

et al., 1991). We believe it unlikely that the declines in T we observed between litters in male *C. kuhlii* reflected the effects of aging rather than acquisition of paternal experience. First, the difference in age among males that varied in infant experience was on the order of months and not years, as in the Ziegler *et al.* (1996) study. Second, while there is age-related variation in urinary T concentrations across the lifetime of male *C. kuhlii*, T concentrations differ little among males during the time course of males used in our study (Boykins, French, and Nunes, unpublished data).

Four of the six males in this part of our study had no paternal experience at the onset of the study, and a fifth male had sired one litter born 1.5 years in the past. The final male in our study was a fifth-time father whose previous litter had been born 2.5 years ago. Because our sample of males was initially composed of novice fathers and fathers without recent paternal experience, it remains possible that experience-related declines in T occur primarily among males at the beginning of their parental careers or among males without recent experience raising young. The most dramatic experience-related differences in endocrine and behavioral variables among mammals in fact occur between primiparous and multiparous females (e.g., Hooley and Simpson, 1981; Holekamp, Nunes, and Talamantes, 1988a,b; Wang and Novak, 1994) and between novice and reproductively experienced males (e.g., Holekamp and Talamantes, 1992).

Experience rearing infants also influenced CORT excretion in male *C. kuhlii*. Urinary CORT levels of males increased after the birth of the first of consecutive litters, but decreased after the birth of the second (Fig. 6C). Thus, variation in CORT between litters differed from variation in T, suggesting that declines in T levels of males between successive litters are not related to CORT or stress. Experience raising young may, however, affect stress responses associated with the birth of infants, with stress responses being dampened among experienced fathers. Another possibility is that postpartum decreases in CORT among male *C. kuhlii* during the rearing of the second of successive litters reflects increased secretion of oxytocin, which decreases CORT secretion and helps promote the formation of social bonds between conspecifics (Carter and Altemus, 1997, but see Cushing and Carter, 2000). Similar postpartum declines in CORT have been observed in human fathers during the period in which they bond to newborn infants (Storey *et al.*, 2000). Experience-related declines in postpartum CORT lev-

els of male *C. kuhlii* might thus be associated with oxytocin-related facilitation of attachment to infants born to a breeding pair with a history of successfully weaning offspring.

We note that although our data are consistent with the hypothesis that paternal experience influences hormone levels in *C. kuhlii*, we cannot fully rule out the possibility that the hormonal changes we observed in males between successive litters were attributable to factors other than experience raising young. If changes in hormone levels between consecutive litters in fact arise from increased paternal experience, then hormone levels of males should change when the appearance of a new litter of offspring is preceded by the successful rearing of young, but not when it is preceded by an unsuccessful attempt to reproduce. Although our current sample did not allow us to make these comparisons, this is clearly a set of observations that would provide a strong test of the hypothesis of experience-mediated changes in hormone levels.

We observed variation in rates of infant-carrying behavior in male *C. kuhlii* between successive litters. Specifically, we there was a trend for hourly rates of infant-carrying among males to decline between successive litters, possibly due to assistance carrying infants provided by offspring of the first litter. Breeding male callitrichids in fact tend to devote less time to carrying infants when residing in groups with many helpers relative to groups with few helpers (Santos *et al.*, 1997). Female callitrichids, by contrast, appear to be more flexible in their infant-carrying behavior, which is influenced by group size as well as other variables (Santos *et al.*, 1997). Because of variation in the size of family groups between litters in our study, we are unable to draw conclusions regarding the link between infant-care behavior and changes in hormone levels associated with acquisition of paternal experience. However, we observed a significant correlation between rates at which male *C. kuhlii* carried infants between the first and second of successive litters. This suggests that individual males are consistent from litter to litter with respect to the amount of effort they devote to caring for offspring, decreasing effort as group size increases due to recruitment of additional helpers.

It is tempting to speculate about the functional significance of experience-related declines in T levels of male *C. kuhlii*. In many monogamous species, individuals seek mating opportunities outside the established breeding pair (Westneat, Sherman, and Morton, 1990; Birkhead and Moller, 1992; Palombit, 1994; Reichard, 1995; Goosens, Graziani, Waits, Farand, Magnolon,

Coulon, Bel, Taberlet, and Allaine, 1998; Spencer, Horsup, and Marsh, 1998). In some normally monogamous males, elevated T levels cause a suite of changes which includes increases in spatial activity, encounters with extrapair females, courtship and copulatory activity, and attractiveness to extrapair females (Chandler, Ketterson, Nolan, and Ziegenfus, 1994; Enstrom, Ketterson, and Nolan, 1997; Raouf, Parker, Ketterson, Nolan, and Ziegenfus, 1997; Kast, Ketterson, and Nolan, 1998). These T-related changes appear to augment reproductive success achieved via extrapair copulations, but decrease reproductive success achieved via caring for young with an established pairmate (Raouf *et al.*, 1997). In *C. kuhlii*, low T levels may cause males who have fathered multiple litters to increase effort devoted to raising offspring with their breeding partners and decrease effort devoted to seeking extrapair mating opportunities. Natural selection might be expected to favor males who invest care in offspring born to an established, successful breeding pair rather than sacrifice infant care to seek outside mating opportunities. Thus, T might help facilitate adaptive shifts in reproductive strategy among male *C. kuhlii* as they acquire paternal experience. In Mongolian gerbils, T does in fact appear to adaptively mediate the existence of alternate male phenotypes differing with respect to the relative amount of effort individuals invest in sexual vs parental behaviors (Clark *et al.*, 1997).

In prior work, we observed that T levels of male *C. kuhlii* temporarily decline while they care for infants, suggesting a link between paternal behavior and T and E_2 (Nunes *et al.*, 2000). Our data in the current study provide converging evidence role for steroid hormones in regulating the paternal behavior of *C. kuhlii*. Our data raise the intriguing possibility that experience-related changes in hormones promote adaptive shifts in reproductive effort toward more extensive investment in raising young fathered with an established breeding partner and less extensive investment in seeking extrapair copulations.

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