

## RESEARCH ARTICLES

### Social and Reproductive Conditions Modulate Urinary Cortisol Excretion in Black Tufted-Ear Marmosets (*Callithrix kuhli*)

TESSA E. SMITH\* AND JEFFREY A. FRENCH  
Nebraska Behavioral Biology Group and Department of Psychology, University of  
Nebraska at Omaha, Omaha

The links between psychosocial stress, social status, reproductive function, and urinary cortisol were assessed in social groups of black tufted-ear marmosets (*Callithrix kuhli*). Urinary cortisol excretion was monitored in cases of intrafamily conflict (“sibling fights”) and in daughters in four distinct social contexts: in the family group, while housed singly or in same-sex pairs, and while paired with a male pairmate. Cortisol excretion was elevated in participants in intra-family conflict on the day of and the day following the conflict, relative to concentrations a week prior to or following the conflict. Daughters in natal family groups had concentrations of cortisol that did not differ from reproductively active adult females. This finding held for daughters who were either anovulatory or undergoing ovulatory cycles while in the natal family group. Natal family members and male pairmates exerted buffering effects on levels of activity in the hypothalamic pituitary adrenal axis (HPA) in female *C. kuhli*. Placing females in solitary housing led to significantly increased cortisol excretion. In the 2 months subsequent to pairing with a male partner, excreted cortisol concentrations in females declined significantly. Daughters removed from their natal family group and housed with a sister did not exhibit increased cortisol levels. These data reveal that activity in the (HPA) axis in marmosets is sensitive to psychosocial stressors, and that urinary cortisol can provide a useful quantitative measure of HPA reactivity. As in other callitrichids, delayed breeding in daughters and reproductive anomalies in *C. kuhli* appear to be mediated by mechanisms other than elevated HPA activity. Am. J. Primatol. 42:253–267, 1997. © 1997 Wiley-Liss, Inc.

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#### INTRODUCTION

Many of the social groupings expressed throughout the primate order (in particular old world primate species) are characterized by dominance hierarchies

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\*Correspondence to: Dr. Tessa E. Smith, Psychology Department, University of Nebraska at Omaha, Omaha NE 68182. E-Mail: tsmith2@cwis.unomaha.edu

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that are established and maintained by agonistic behavioral interactions [see review in Smuts et al., 1987]. These interactions can have profound physiological consequences, since several studies examining links between social status and HPA activity suggest that dominant animals typically display low adrenocortical output, a rapid adrenocortical response to a stressor, and high sensitivity to negative feedback regulation resulting in a rapid return of cortisol to basal levels [Manogue et al., 1975; Keverne et al., 1982; Sapolsky, 1983a, 1993; but see Saltzman, et al., 1994; Ziegler et al., 1995]. As a result, dominant individuals tend to be spared the detrimental consequences of a prolonged stress response such as impaired immune function and reproductive suppression [Rivier & Rivest, 1991; Coe, 1993]. The life of a low-ranking primate, on the other hand, is one of unpredictability, minimal control and a weak social support system—the typical hallmarks of stress [Levine, 1993]. Under conditions of group stability, subordinate individuals tend to have increased activity in the HPA axis relative to dominant individuals [see review in Sapolsky, 1993]. Indeed, many accounts of reduced fertility in subordinate primates (particularly old world and hominoid primates) attribute the low reproductive output in subordinate individuals to stress associated with their low social rank [Wasser and Barash, 1983; Adams et al., 1985; Kaplan et al., 1986; Perret, 1986; Harcourt, 1987; Dunbar, 1989; Rivier & Rivest, 1991]. In contrast to the demonstrated link between HPA activity and social status in old world primates, however, recent studies have found no link between social status and levels of activity in the HPA axis in a variety of species [e.g., Schoech et al., 1991; Saltzman et al., 1994; Ziegler et al., 1995; Faulkes & Abbott, 1996].

Among neotropical primates, the callitrichids (marmosets and tamarins) exhibit dramatic rank-related differences in endocrine function, copulatory behavior, and, presumably, reproductive success [see reviews in Abbott, 1993; French, 1996]. Subordinate females in groups of unrelated peers, and post-pubertal daughters residing in their natal family group, exhibit socially-mediated endocrine and behavioral suppression of reproduction to the extent that, in some callitrichid species, subordinate females (peer groups) and daughters (family groups) are rendered anovulatory [Abbott et al., 1981; French et al., 1984; Ziegler et al., 1987]. More recent evidence suggests that subordinate males and sons in social peer groups and natal family groups, respectively, also experience endocrine and behavioral suppression, since levels of testosterone are lower in these males than in dominant breeding males [Abbott et al., 1992; French & Schaffner, 1995]. Thus, the callitrichids would appear to be good candidates to test the role of status-related HPA function in mediating differences in reproductive potential.

The potential link between stress-induced activation of the HPA system and reproductive suppression in subordinate female marmosets and tamarins has been addressed in several studies. In groups consisting of unrelated male and female common marmosets (*Callithrix jacchus*), there was a dramatic suppression of ovarian function in subordinate females, but plasma cortisol levels did not differ between cycling dominant and acyclic subordinate females [Abbott et al., 1981]. More recently, Saltzman et al. [1994] demonstrated that plasma cortisol concentrations were higher in dominant females than in subordinates. However, elevated cortisol in dominants was related more to reproductive status (i.e., whether or not the female was undergoing regular ovulatory cycles) than to social status. Ovulatory status, and not social status, was the best predictor of elevated cortisol concentrations in female common marmosets [Saltzman et al., 1994]. Likewise, in cotton-top tamarins (*Saguinus oedipus*), relative levels of HPA activity are not consistent with a “stress-induced” reproductive suppression hypothesis.

Urinary cortisol excretion in anovulatory (but post-pubertal) daughters housed in their natal family group is significantly lower than levels of excreted cortisol in the same females while housed with or near an unrelated male and undergoing normal ovulatory cycles [Ziegler et al., 1995].

Although the link between increased adrenocortical activity and psychosocial stressors is clear for a variety of primates [e.g., Eberhart et al., 1980; Anzenberger et al., 1986; Mendoza & Mason, 1986; Sapolsky, 1990], there is only minimal information in the literature on the responsiveness of the callitrichid HPA system to psychosocial stressors. In fact, some protocols for assessing HPA activity (e.g., measurement of urinary cortisol in first-morning void samples) may not be effective for monitoring the consequences of acute or chronic psychosocial stressors. In cotton-top tamarins, concentrations of urinary cortisol in samples collected during periods of capture and restraint for blood sampling did not differ from concentrations in samples collected from unstressed animals [Ziegler et al., 1995]. Saltzman et al. [1994] demonstrated that plasma cortisol was elevated in participants in fights that involved wounding, but in this context it is difficult to separate HPA activation associated with physical wounding from the psychosocial components of conflict. Our previous work [Smith & French, in press] demonstrated that concentrations of urinary cortisol in marmosets are elevated in response to relatively mild but acute stress (e.g., short-term hand-held restraint), but there has been no demonstration in callitrichid primates of elevated HPA activity associated with either chronic or acute episodes of psychosocial stress.

In the present paper, we evaluated the association between HPA activity, intra-family conflict, social status, and reproductive function in female black tufted-ear marmosets (*Callithrix kuhli*). In the first study, we examined the utility of urinary cortisol excretion as a measure of psychosocial stress in a context distinct from reproductive function. Sibling conflicts within family groups ("sibling-fights") occur commonly in marmoset and tamarin social groups [Kleiman, 1979; Sutcliffe & Poole, 1984; McGrew & McLuckie, 1986; Rothe et al., 1988; Inglett et al., 1989] and while rarely injurious, these conflicts constitute a significant source of social disruption within groups. We wished to test, therefore, whether known or suspected psychosocial stressors such as sibling fights could produce changes in cortisol excretion.

In the second study, we compared cortisol excretion in daughters in four contexts: 1) in the natal family group, 2) after removal from the natal group and housed alone or 3) with a sister, and 4) after pairing with an unrelated adult male. *C. kuhli* provide a particularly useful model for distinguishing between changes in cortisol induced by subordinate-related stressors versus those changes produced by the onset of normal ovarian activity. Social factors appear to exert inhibitory influences on the reproductive functioning of young female *C. kuhli* residing in the presence of the dominant breeding female, but these effects are manifest in two ways. First, young daughters (i.e., less than 15 months of age) are anovulatory but can commence ovulation immediately upon removal of the dominant breeding female [Smith et al., in press]. In contrast, older daughters commence ovulatory function around 15 months of age while still living with their family, but social factors still appear to exert an influence on reproductive physiology. The ovarian cycles of older daughters are significantly different from those of breeding females and are characterized by short luteal phases and reduced concentrations of the progesterone metabolite, pregnanediol 3- $\alpha$ -glucuronide [Smith et al., in press]. Therefore, with *C. kuhli* we have the possibility of contrasting cortisol excretion profiles of both ovulatory and anovulatory daughters housed with a breeding female with those of ovulating breeding females.

## MATERIALS AND METHODS

### Subjects

The study involved 15 female and 3 male black tufted-ear marmosets housed in six family groups at the Callitrichid Research Facility at the University of Nebraska at Omaha. All marmosets were raised in their natal group until at least 12 months of age. Each family group contained only one breeding female. Animals were housed in cages measuring 1.6 × 0.9 × 2.4 m (family group) or 1.6 × 0.9 × 0.9 m (pairs and singly housed animals), equipped with natural branches, one or two feeding platforms, a nest tube, and enrichment devices. No more than two family groups or four pairs were housed in a single colony room. Marmoset groups within a colony room were in olfactory and auditory contact with neighbors but were denied direct visual contact. Animals were fed a varied diet twice daily, and experienced a 12h:12h light:dark cycle, with the light period spanning 0700–1900 h. Temperature was 22–25°C and relative humidity was maintained between 45–55% [for further husbandry details, see Schaffner et al., 1995].

### Urine Collection

The first void urine sample of the day was collected in a noninvasive, stress-free manner from subjects in their normal social group 4–6 days a week, commencing when the marmosets were approximately 30 days of age. All animals had been trained to urinate for a desired food item and would typically urinate within 20 min of waking. Void urine fell onto large stainless steel pans or was caught in hand-held aluminum pans. Urine samples were centrifuged at 700 rpm for 2.5 min and the supernatant transferred to a microcentrifuge tube. A 300 µl aliquot destined for LH analysis was mixed with glycerol (1:7 glycerol: distilled water; 10 µl glycerol solution per 100 µl urine) to prevent cryodegradation [Liversey et al., 1983]. Urine samples were stored at -20°C until assayed.

### Assessing Female Reproductive Condition and HPA Activity

Female reproductive condition was assessed by measuring excreted concentrations of the progesterone metabolite pregnanediol 3- $\alpha$ -glucuronide (PdG) and luteinizing hormone/chorionic gonadotropin (LH/CG). Concentrations of urinary PdG were determined using an enzyme immunoassay (EIA) previously characterized by Munro et al. [1991] and adapted for *C. kuhli* as reported by French et al. [1996]. Assay sensitivity at 90% binding was 78 pg. Displacement curves of halving dilutions of urine pools from pregnant females were parallel in the 10–90% binding range. Recovery of standards (10,000, 1,250, and 78 pg) added to the pregnant female urine pool was 110.7%. Intra-assay coefficients of variation for low and high concentration pools were 6.1% and 7.3% (n=22), respectively. Inter-assay coefficients of variation for low and high concentration pools were 18.2% and 14.1% (n=22), respectively. Concentrations of urinary gonadotropin were measured using a LH/CG radio-immunoassay previously characterized by Matteri et al. [1987; see also Ziegler et al., 1993] and validated for use in *C. kuhli* [French et al., 1996]. Intra-assay coefficients of variation based on repeated measure of low and high concentration urine pools were 15.3% and 9.9% (n=6), respectively. Inter-assay coefficients of variation for low and high concentration urine pools were 19.9% and 15.7% (n=11), respectively.

Activity in the HPA axis was estimated by measuring concentrations of urinary cortisol. We used an EIA validated for *C. kuhli* and described previously by

Smith and French [in press]. Recovery of ten standards (range 1.95–1,000 pg) added to a low and medium concentration urine pools was  $101 \pm 2\%$ . The intra-assay coefficients of variation for high and low concentration pools were 4.5 and 2.8%, respectively (n=28). Inter-assay coefficients of variation for high and low concentration pools were 17.2% and 18.2%, respectively (n=28).

To control for variations in fluid intake and output, hormone concentrations were corrected for the creatinine concentration of each sample. Creatinine concentration was measured by a modified Jaffé end-point assay [Tietz, 1976].

### Sibling Fights

Intrafamily conflict among siblings is a well-known, but relatively rare, occurrence in callitrichid social groups [Kleiman, 1979; McGrew & McLuckie, 1986; Inglett et al., 1989]. In order to be considered as having participated in a sibling fight, participants had to be observed engaged in wrestling, chasing, and attempted or actual biting. The reported sibling fights occurred between 0800–1700 h, and resulted in only minor physical wounds. Four sibling fights were examined in this study. Two of the fights were within female–female twin pairs (ages: 13.2 and 15.5 months, respectively) and two of the fights were within pairs of same-sex non-twin siblings (female sibling pair, 13.2 and 18.2 months of age, and male sibling pair, 16 and 26.3 months of age). Since all fights were spontaneous occurrences systematic behavioral data directly associated with the fight was not available. All fights were signaled by obvious vocalizations (e.g., “erh-erh”) and screams audible to researchers in adjoining offices. All fights reported in this manuscript were physically terminated within approximately 3 min.

We were able to monitor urinary cortisol in both participants in the male–male and female–female non-twin sibling fight. Samples of urinary cortisol were only available for one of the participants in each of the female–female twin fights. Concentrations of urinary cortisol were measured in the four females and two males (the fighters) in four periods surrounding a sibling fight: i) 7, 8, and 9 days immediately prior to a sibling fight, ii) the day of and iii) day following a sibling fight, and iv) 7, 8, and 9 days following the fight. Since concentrations of urinary cortisol were always determined in the first void sample of the day, urinary cortisol concentrations the day of the fight probably reflect levels of circulating cortisol on the day and evening prior to the observed fight. To control for extraneous environmental factors that might have increased HPA activity around the time of the fight, concentrations of urinary cortisol were measured over the same time periods in siblings that were in the group at the time of the fight but did not participate in the agonistic interactions (n=4). Siblings whose age was closest to that of the fighters were used as control animals. The age difference between fighters and control siblings was not greater than 6 months (age range 7–21 months). For the six fighters and four control animals, concentrations of urinary cortisol were averaged 7, 8, and 9 days prior to and following the fight to provide a single pre- and post-fight cortisol value for each animal.

### Association Between HPA Activity and Female Reproductive Status

Eleven females were used to assess relationships between HPA activity and female reproductive status. Hormone concentrations (PdG, LH/CG, and cortisol) were measured in the following groups of females for the respective time period as indicated.

- 1) Juveniles: Females (aged 4–5 months inclusive) in the natal group (n=8). Each female was monitored for 2 months.

- 2) Anovulatory daughters residing in the family group: Females had never ovulated while residing in the natal group. Data for analysis was drawn from the 2 month period when these females were aged 13 and 14 months of age (n=9).
- 3) Daughters in the family group that commenced ovulatory function: Older daughters in family groups ( $16.4 \pm 1.1$  months of age) commenced ovulatory function while still residing as a daughter in the natal group (see Results). Urine samples were collected during a 6 week period for each female, which commenced 2 weeks following the onset of ovulatory function (n=6).
- 4) Established breeding females: Endocrine excretion was monitored in six breeding females residing with a long-term male pair-mate and one to four offspring (age range 2.7 to 7.1 years of age) through at least one complete pregnancy cycle (mean period parturition to parturition was  $148.17 \pm 1.14$  days; n=6).

### Association Between HPA Activity and Female Social Condition

To assess the relationship between HPA activity and changes in social conditions, urinary cortisol excretion in daughters housed in their natal family groups (see above) was contrasted with cortisol excretion in the following situations:

- 1) Singly housed females: Five daughters (age range: 16–22 months old) were removed from the family group and housed alone. Concentrations of urinary cortisol were monitored for 3–8 weeks.
- 2) Iso-sexually housed females: Three daughters (age range 13–16 months old) were removed from the family group and housed in an iso-sexual pair with a sister that was either their twin (n=2) or younger sibling (n=1). Urinary cortisol was measured for 12–32 weeks.
- 3) Heterosexual pair: Females that had been housed alone (n=5) or in an iso-sexual pair (n=3) were paired with an unfamiliar male. Urinary cortisol was assessed during the first and second month of pairing (n=8). Female age ranged from 14.1 to 22.3 months old.

For each individual, multiple samples (i.e., 4–6 per week) were analyzed for each respective time period and a mean concentration of urinary cortisol determined. Mean concentrations of urinary cortisol for each female served as the data points in analyses.

## RESULTS

### Sibling fights

Urinary cortisol concentrations in urine samples of fight participants collected approximately a week prior to the fight, the day of and day after the fight, and approximately 1 week after the fight were analyzed by one factor ANOVA. Cortisol excretion differed significantly across these sampling periods [ $F(3,15) = 7.8$ ;  $P < 0.01$ ; see Fig. 1]. Post-hoc analyses, using Tukey HSD, showed that cortisol was excreted in significantly higher concentrations the day of and the day following the fight than excreted concentrations a week before and a week following the fight ( $P$ 's  $< 0.05$ ). Cortisol concentrations were elevated on the day of the fight and did not drop significantly on the day following the fight. There was no difference between cortisol values a week prior to and a week following the fight. The magnitude of the urinary cortisol response to fighting did not vary with the relative success of participants in the fight. On the day of the fight,

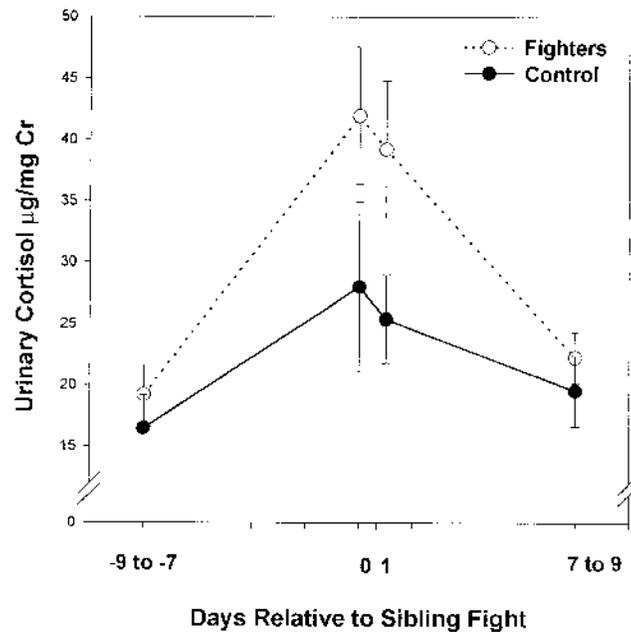


Fig. 1. Mean urinary cortisol values  $\pm$  s.e.m. for animals directly involved in sibling fights (Fighters,  $n=6$ ) and other age-matched group members not directly involved in physical aggression (Control,  $n=4$ ). Data are presented relative to the day of the observed sibling fight (Day 0).

there were no difference in cortisol levels between the fighter that directed most aggression during that fight and the fighter that received most aggression during the fight ("aggressors":  $42.4 \pm 6.5$   $\mu\text{g}$  cortisol/mg Cr; "losers":  $41.5 \pm 10.7$   $\mu\text{g}$  cortisol/mg Cr). Unlike participants in the fights, control siblings in the family group showed no change in cortisol concentrations a week prior to the fight, the day of and day after the fight, and one week after the fight [ $F(3,10) = 1.3$ , NS].

#### Association Between HPA Activity and Female Reproductive Status

In terms of reproductive endocrine status, juvenile females aged 4–5 months had low acyclic levels of urinary PdG ( $1.4 \pm 0.2$   $\mu\text{g}/\text{mg}$  Cr) and LH/CG ( $4.2 \pm 0.6$  ng/mg Cr). Daughters aged 13–14 months residing in their family group also had low acyclic levels of urinary PdG ( $1.9 \pm 0.4$   $\mu\text{g}/\text{mg}$  Cr) and LH/CG ( $4.2 \pm 0.7$  ng/mg Cr) suggesting anovulation. At a mean age of  $16.4 \pm 1.1$  months (range: 14.1–21.5 months), levels of urinary PdG rose dramatically in six daughters to luteal phase concentrations [between 10 and 60  $\mu\text{g}$  PdG/mg Cr; see French et al., 1996] for extended periods of time (mean duration of raised PdG levels was  $13.6 \pm 1.8$  days). Hormone profiles for these females showed LH spikes prior to the rise in PdG levels, confirming that ovarian function was organized into ovulatory events. The dynamics of the ovarian cycles exhibited by these daughters, however, were characterized by significantly short luteal phases and reduced PdG concentrations compared to the ovarian cycles of adult breeding females [see Smith et al., in press, for details]. Breeding females exhibited hormone profiles throughout the pregnancy as reported previously by French et al. [1996] and Smith et al. [in press].

There was a significant effect of female reproductive status on cortisol levels

that was caused by significantly elevated cortisol levels in breeding females during the third trimester of pregnancy [ $F(7, 45) = 12.7$ ;  $P < 0.001$ ; Fig. 2]. Post-hoc analyses revealed no significant difference between cortisol levels in daughters (juvenile, anovulatory and ovulatory) and breeding females during all stages of pregnancy except for the third trimester. Cortisol levels in females during the third trimester were significantly higher than cortisol levels in females in all other reproductive states ( $P < 0.01$ ).

### Association Between HPA Activity and Female Social Condition

The social condition under which females were housed had a significant effect on concentrations of urinary cortisol, but only for females that were housed alone prior to pairing with a male [ $F(3,12) = 20.65$ ;  $P < 0.001$ ; see Fig. 3a]. Post-hoc analyses revealed that cortisol excreted by females while housed alone was significantly higher than while housed in their family group. In addition, levels were significantly reduced during the first month of pairing with an unrelated male ( $P < 0.05$ ), and were further reduced during the second month of cohabitation ( $P < 0.05$ ). Levels of excreted cortisol during the second month of pairing were not significantly different from those while the female was housed in the natal family group. In contrast, removing a female from the natal family group and housing her with a sister led to only slight elevations in urinary cortisol, and the changes across social conditions were not significant [ $F(3,6) = 1.62$ , NS; see Fig. 3b].

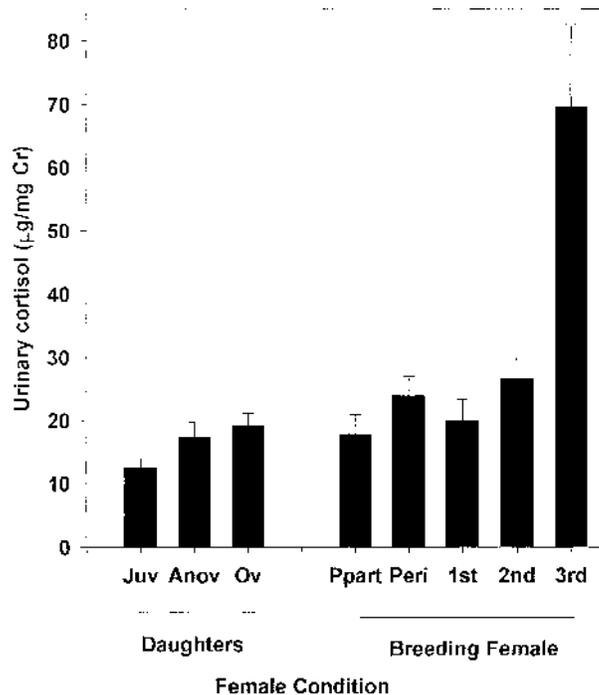


Fig. 2. Concentrations of urinary cortisol (mean  $\pm$  s.e.m.) in daughters and established adult breeding females. Juv, Juvenile in natal group (n=8); Anov, Anovulatory daughter in natal group (n=9); Ov, Ovulatory daughter in the natal group (n=6); Ppart, post partum period (n=6); Peri, periovulatory period (n=6); 1st, first (n=6); 2nd, second (n=6); 3rd, third trimester of pregnancy (n=6).

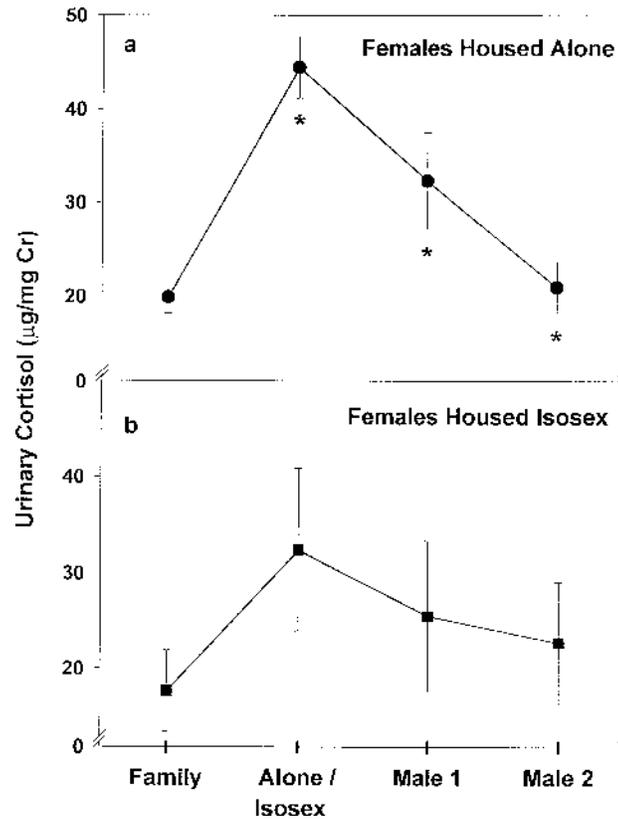


Fig. 3. Mean concentrations  $\pm$  s.e.m. of excreted urinary cortisol in females housed in various social conditions: **a**: females housed in natal family group (Family), removed from natal group and housed singly in a cage (Alone), and during each of the first 2 months of housing with a male (Male 1 and Male 2;  $n = 5$ ); **b**: females in the same conditions as in **a**, with the exception that upon removal from the natal family group, females were housed with a sister from their natal family group (Isosex;  $n = 3$ ). \*, cortisol concentrations significantly different ( $P < 0.05$ ) from previous sampling phase.

## DISCUSSION

The study has established that the measurement of urinary cortisol can be used as a valid indicator of psychosocial stress in the marmoset. These findings extend our earlier study that showed urinary cortisol to be a sensitive, quantitative measure of HPA reactivity to stressors, such as short-term manual restraint, in marmosets [Smith & French, in press]. The present study has shown that naturally occurring family events such as sibling fights, which probably constitute a major source of psychosocial stress, can be potent stimulants of HPA activity. One of the most striking results to emerge from the study was that reproductive anomalies in daughter marmosets living with their family (i.e., anovulation in younger daughters and significantly impaired ovarian cycles in older daughters) was apparently not related to activation of the HPA axis: there were no differences in cortisol levels in anovulatory, and ovulating daughters residing in the family group, and cortisol concentrations in both these groups of females were comparable to levels in adult breeding females. Finally, the study has hinted at the important links between social environment and HPA activity in marmo-

sets, especially the potential for social partners to modulate the magnitude of HPA responses to stressors.

Marmosets involved in sibling fights had significantly increased levels of urinary cortisol the morning of the day of the fight and the day following a fight. In contrast, similar-aged siblings in the family group did not exhibit increased cortisol levels around the time of the fight. This suggests that family members not involved in the fight were not affected by the social instability occurring between the two participants [see also, Saltzman et al., 1994]. Fight participants had increased urinary cortisol immediately prior to the fight suggesting that the fight was preceded by escalating social tension. Elevated cortisol levels in fighters prior to the fight also suggest that social tension and instability in social relations alone, in the absence of physical aggression, are potent stimulants of HPA activity in *C. kuhli*. We cannot discount, however, the possibility that an undocumented fight could have occurred prior to the observed fight that might have contributed to elevated cortisol. The clear absence of physical wounds on animals prior to the fight, however, is not compatible with this possibility. The emotional threat of being attacked can constitute a major source of stress [Bronson & Eleftheriou, 1963; Chamove & Brown, 1978]. Primates often exhibit increased HPA activity in the face of social instability, including group formation [Chamove & Brown 1978; Mendoza et al., 1979; Coe et al., 1979, 1982; Keverne et al., 1982; Sapolsky, 1992; but see Mendoza & Mason, 1989; Gust et al., 1993] and hierarchy reorganization within established groups [Sapolsky, 1983b; McGuire et al., 1986]. In callitrichid primates, sibling fights represent a dramatic source of social instability, and, as in other species, they are associated with elevated cortisol.

#### **Association Between HPA Activity and Female Reproductive Status**

Our study has shown, quite clearly, that anovulation in younger daughters, and impaired ovarian cycles in older daughters, are not mediated via stress-related hypercortisolism in female *C. kuhli*. If anovulation or luteal insufficiency was mediated via this mechanism, we would have expected cortisol levels to be higher in daughters than in adult breeding females. This outcome was not observed. Instead, both categories of daughters living in their family group had cortisol levels that were not different from cortisol levels in nonpregnant or early-pregnant breeding females. Two recent studies also demonstrated that levels of cortisol were not significantly higher in anovulatory (pair-housed and peer-group housed) female common marmosets, and daughter cotton-top tamarins, than in dominant breeding females, suggesting that stress-induced HPA activity is not the major proximate cause of reproductive suppression in female callitrichids [Saltzman et al., 1994; Ziegler et al., 1995]. Levels of aggression are low in callitrichid groups, implying that subordinate individuals are not constantly exposed to stress-inducing behavioral harassment and aggression, as are subordinate Old World primates [Caine, 1993; Price & Hannah, 1983; Coates & Poole, 1983; Schaffner, 1991]. Our observations are consistent with reports from other cooperatively breeding species, both mammalian and avian, that reproductive suppression in subordinates is not mediated via stress-associated HPA activity [Corbett, 1988; Schoech et al., 1991; Faulkes & Abbott, 1996; van Jaarsveld & Skinner, 1992].

In the present study, cortisol levels did not differ between cycling daughters and acyclic daughters. This is in contrast to previous studies in callitrichid primates that have reported higher cortisol titers in cyclic versus acyclic individuals [Saltzman et al., 1994; Ziegler et al., 1995]. The contrasting results might be

related to the fact that the ovarian cycles exhibited by daughters in *C. kuhli* residing in the family group are significantly impaired compared to those exhibited by adult breeding females [Smith et al., in press]. The pattern of impaired ovarian activity observed in subordinate *C. kuhli* has also been observed in common marmosets and termed oligocyclic by Saltzman et al. [1994]. Oligocyclic female common marmosets had cortisol values that were intermediate between those of cycling and acyclic females (high and low levels, respectively) but did not differ significantly from either group—a result similar to that observed in the present study [Saltzman et al., 1994].

### Female Social Condition and HPA Activity

The presence of a familiar social partner can significantly buffer an individual against stress [see review by Levine, 1993]. The “social buffering” or “social support” effect [as defined by Cohen & Wills, 1985] can significantly reduce the adrenocortical correlates of stress induced by a host of social and physical stressors in primates such as environmental novelty and disrupted species-specific relations among adults; both of which were experienced by subjects in our study [e.g., Weiner et al., 1990; Mendoza et al., 1991; Gust et al., 1994; Hennessy et al., 1995]. Our results suggest that two kinds of social partners can exert important regulatory, or buffering, effects on HPA activity in female marmosets. First, natal family group members may serve an important role in lessening the impact of stressors on marmosets. Removing females from their family group and housing them alone produced dramatic increases in urinary cortisol, a rise that was not observed when females were removed from their family group and housed with a sister. When females were housed alone, they were isolated from significant social partners and housed in an unfamiliar environment, both of which are potent stimulants of HPA activity in primates [e.g., Cubicciotti et al., 1986; Mendoza et al., 1992; Hennessy et al., 1995; Ziegler et al., 1995]. The dampened HPA response to removal from the family group and housing in unfamiliar conditions suggests that natal family members can constitute social buffers in marmosets. Alternatively, increased HPA activity upon isolation might have been caused solely by the loss of homeostatic regulatory mechanisms provided by familiar social partners, rather than a “stress” response produced by exposure to novel housing conditions. Females housed in iso-sexual pairs were still exposed to homeostatic buffers and would not, therefore, have been expected to demonstrate increased cortisol levels.

The second class of social partner that appear to regulate HPA activity in marmosets is a heterosexual partner, or long-term pairmate. In this study, placing females with an unfamiliar male following a period of single housing was associated with a significant decrease in levels of urinary cortisol, an effect that continued into the second month of cohabitation with the male. Decreasing cortisol levels in females might, however, have been caused by female habituation to the isolation conditions, which happened to coincide with the introduction of a male partner. The present study is unable to distinguish between these two hypotheses. The decline in cortisol levels observed in the present study, either in response to the introduction of a male or as part of a habituation process, is in contrast to results observed in cotton-top tamarins and squirrel monkeys. In these two species, cortisol levels did not change in females following prolonged isolation and introduction of a male partner [Ziegler et al., 1995; Mendoza & Mason, 1989; Mendoza et al., 1991]. Our results suggest that the continuous presence of a male partner may exert

down-regulatory effects on HPA activity in marmosets. Since pair-related social relationships are fully expressed by 60 to 80 days post-pairing [Schaffner et al., 1995], these results suggest that heterosexual social relationships in marmosets might function as significant homeostatic regulators of HPA activity. There is considerable individual variability in the pace with which heterosexual social relationships develop in marmosets, and in the "quality" of the pair relationship, as indexed by affiliative social interactions [Schaffner et al., 1995; Schaffner, 1996]. A good test of the relationship we propose here between HPA function and social interactions with a heterosexual partner would be to assess the correlation between urinary cortisol and relationship quality. In any event, since prolonged elevations in circulating cortisol have severe, deleterious effects on physiological functioning [Rivier & Rivest, 1991; Coe, 1993], our study illustrates the importance of social relationships to the well-being of marmosets.

## CONCLUSIONS

1. Marmosets directly involved in naturally occurring sibling fights had significantly elevated excreted cortisol concentrations the day of the fight and the following day, relative to cortisol levels a week prior to and a week subsequent to the fight. Since cortisol levels were elevated in the morning sample collected prior to the outbreak of the fight, social tension, and group instability alone, in the absence of wounding or physical aggression, appear to be potent stimulants of HPA activity in marmosets.

2. Cortisol concentrations did not differ among juvenile females, anovulatory or ovulatory daughters, and breeding females throughout all stages of pregnancy except the third trimester. This finding established that anovulation and impaired ovarian functioning in sub-adult daughters is not mediated via activation of the HPA axis.

3. Natal family members and heterosexual partners appear to exert significant buffering effects on HPA activity in female *C. kuhli*. Removing daughters from the family group and housing them alone produced significant increases in levels of urinary cortisol. Pairing these same females with an unfamiliar male led to a decrease in cortisol concentrations. Daughters removed from their natal family group and housed with a sister did not exhibit increased cortisol levels.

4. The present study has demonstrated the validity of urinary cortisol as a sensitive quantitative measure of psychosocial stress in marmosets, since cortisol concentrations increased during times of intrafamily conflict and during social isolation. In addition, the study has established that socially-modulated reproductive anomalies in *C. kuhli* are mediated by mechanisms distinct from the HPA axis.

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