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Psychosocial Stress and Urinary Cortisol Excretion in Marmoset Monkeys (*Callithrix kuhli*)

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SMITH, T. E. AND J. A. FRENCH. *Psychosocial stress and urinary cortisol excretion in marmosets* (*Callithrix kuhli*). PHYSIOL BEHAV **62**(2) 225–232, 1997.—Activation of the hypothalamic–pituitary–adrenal (HPA) axis is one of the hallmarks of the physiological responses to psychosocial stressors. The most common method of assessing HPA function is via the measurement of plasma cortisol levels. However, venipuncture involves capture and restraint, which can modify HPA function. We validated a noninvasive procedure for monitoring HPA responses to stressors by measuring excretion of free urinary cortisol. Samples collected throughout the day displayed marked circadian variation, with low cortisol values in first–void samples, followed by a mid–morning peak in cortisol excretion. Concentrations of excreted cortisol declined throughout the day. Exposing marmosets to mild and moderate stressors (11 h isolation in a small cage and manual restraint) increased excreted cortisol concentrations in a dose–dependent fashion: isolation in a small cage led to elevated cortisol in afternoon samples, while manual restraint and isolation produced elevated cortisol in both morning and afternoon samples. The marmoset HPA is differentially sensitive to rather subtle variations in stressors, and these results show that urinary cortisol excretion is a valid and sensitive index of the HPA response to these stressors. © 1997 Elsevier Science Inc.

Stress Marmosets HPA axis Urinary cortisol Separation *Callithrix kuhli* Circadian rhythm

EXPOSURE to a stressor (physical or psychological), produces a host of physiological processes (i.e. 'the stress response'), that enable the animal to cope with, or survive, the stressor (e.g. increased heart rate, respiration and metabolism). One of the prominent physiological changes in response to a stressor is increased activity in the hypothalamic–pituitary–adrenal (HPA) axis and concomitant increases in circulating glucucorticoids, together with increased activity in the sympathetic nervous system.

Although many endogenous factors modulate the magnitude of the stress response, there is increasing evidence that factors exogenous to the individual can dramatically affect the nature, duration and severity of the physiological consequences of exposure to stressors [reviewed in (44)]. Primates provide excellent models to investigate the links between the endocrine correlates of psychosocial and physical stress, since social factors have been shown to both potentiate and alleviate the physiological response to stressful stimuli in primates [e.g., reviewed in (11)]. Many primate groupings (e.g., matrilines, coalition alliances, bachelor groups, harems) are characterized by dominance hierarchies established and maintained by aggressive behavior, thus social relationships constitute potent promoters of HPA activity (23,59,61). At the same time, however, the complex network of affiliative social relationships within a group provide an important social support system that protects an animals against the deleterious consequences of stress [''social buffering'' or ''social support'' effect; reviewed in (11,36)] (27,30,70).

Concentrations of plasma cortisol have been widely used to quantitatively assess the stress response in animals [e.g. 7,27,30,57)]. However, obtaining a blood sample from an animal by venipuncture usually involves capture, restraint, and possibly anesthetization. All of these procedures have been shown, in some species, to significantly increase HPA activity and therefore potentially mask, or artificially potentiate, responses to experimental treatments (5,13,29,32,58). In addition, separation of group–living subjects for sample collection is not an ''experimentally neutral'' procedure, since it requires temporary isolation from familiar social partners and familiar surroundings, both of which promote increased HPA activation (28,30,51). One of the major metabolic fates of plasma cortisol, like other steroid hormones, is excretion in urine (38). Urinary cortisol concentrations thus represents a potential method for assessing HPA activity utilizing less disruptive sample collection procedures. The use of urinary cortisol as an index of response to stressors has been utilized in studies of both human (17,43) and

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non–human primates (13,32). One potential shortcoming of this method regards the temporal resolution between exposure to a stressor and excretion of associated cortisol in the urine. In the cotton–top tamarin (*Saguinus oedipus*), the clearance rate of infused, radioactive labeled steroids, ^{14}C–estrone, ^{14}C–estradiol, and ^{14}C–progesterone, was relatively slow (i.e. time from injection to the recovery of the highest concentration of each labeled steroid was 48, 8 and 24 h respectively; 73). A recent evaluation of metabolic clearance rates of cortisol in human patients, however, suggests that cortisol is excreted relatively quickly in urine. The half–life of IV infused [^{3}H]–labeled cortisol in the circulation was 30 min, and the maximal excretion of label in the urine occurred 1.8 h post–infusion (41). The relatively fast clearance rate of cortisol in the circulation suggests that urinary cortisol might provide a good index of HPA activity and stress.

The callitrichid primates—marmosets and tamarins—are good models in which to investigate stress socioendocrinology. Marmosets live in small groups of 5 to 15 individuals which are relatively permanent over time, and maintain complex social relationships (56). There is a strong reliance on within–group cooperation (e.g., cooperative care of the young, territory defense, foraging, anti–predator behavior) and strong heterosexual attachments (4,26,40,62,65). There are also, however, forces within the group that promote social stress and instability. Reproduction is typically restricted to a single breeding pair, with physiological suppression of reproduction imposed on the other individuals (24). Breeding exclusion constitutes a major force promoting intra–sexual competition for breeding opportunities (21,31,50). The aim of the study was to validate the use of urinary cortisol as a non–invasive, reliable and sensitive measure of stress in marmosets. There were three main goals to the study. First, we wanted to see if urinary cortisol excretion throughout the day would accurately reflect the well–established circadian rhythm of cortisol production (18,47). A second goal was to investigate whether exposing marmosets to a stressor produced reliable increases in concentrations of urinary cortisol and to assess the temporal relationship between exposure to a stressor and subsequent changes in cortisol excretion. The third aim of the study was to test whether the marmoset HPA axis was sensitive to the relative severity of the stressor. To the extent that urinary cortisol is a valid index of HPA response to stressors in marmosets, we predicted significantly higher concentrations of urinary cortisol following a major stressor relative to a minor stressor.

METHOD

Subjects

Subjects were seven male and nine female Wied's black tufted–ear marmosets aged 1.2 to 10.2 years, housed in social groups at the University of Nebraska at Omaha Callitrichid Research Facility (see Table 1). Marmosets were housed in either family groups or breeding pairs or trios that had been established for at least six months. Six of the females used in the study were breeding females in the first or second trimester of pregnancy. The remaining three females were the eldest daughter in the family group, and were all exhibiting ovulatory cycles. Levels of urinary cortisol are at baseline concentrations in females during these reproductive states (Smith and French, in press). Of the seven male subjects, one male held breeding position in a family group, five males were housed in two male:one female trios, and one male was the oldest son in a family group. Marmosets were housed in large cages measuring 1.6 × 0.9 × 2.4 m, equipped with natural branches, a feeding platform, a nest tube and several enrichment devices. Cages containing neighboring groups or pairs were at least 1 m apart and adjoining groups were denied visual contact. Our routine husbandry practices and experimental protocols involved little direct handling or disruption of animals. Removing animals from the family group was achieved in a non–stressful manner by enticing the animal into a small transport cage (30 × 32 × 34 cm) attached to the home cage. Animals were housed undisturbed in their family or social group throughout the duration of the experiment and experienced no obvious stressors other than the experimental manipulations described in this study. Animals were fed a varied diet twice daily and water ad lib [see (62) for details]. Animals experienced a 12 h:12 h L:D cycle, with light onset at 0600 h. Room temperature was maintained at 22–24°C and relative humidity was 45–55%.

TABLE 1

SUBJECT IDENTIFICATION AND DETAILS

Sex	Subject Identification	Age (years)	Housing	Status
Females	Ang	7.1	Family	Breeder
	Bon	3.9	Family	Breeder
	Cor	10.2	Family	Breeder
	Reb	6.1	Family	Breeder
	Oli	2.5	Pair	Breeder
	Pix	2.0	Pair	Breeder
	Wir	1.2	Family	Sub-adult
	Una	1.2	Family	Sub-adult
	Yaz	1.1	Family	Sub-adult
Males	Ell	3.2	Polyandrous group	Breeder
	Ken	3.3	Polyandrous group	Breeder
	Moz	2.7	Polyandrous group	Breeder
	Att	4.5	Pair	Breeder
	Geo	4.6	Family	Breeder
	Ren	1.8	Family	Sub-adult
	Zip	1.1	Family	Sub-adult

Experimental Design

All subjects were exposed to three conditions: a manipulation–free condition (control) and two conditions that we assumed to constitute more or less severe stressors for our animals, bearing in mind our non–invasive husbandry and research protocols. At least one month separated exposure to the three conditions.

Control. Animals were housed undisturbed in their home cage with their normal social or family group. The control day for 4 males and 4 females immediately preceded one of the stressor conditions, and the control day for the remaining 8 subjects preceded the other stressor condition.

Small Cage (SC). After collection of the first void sample of the day, subjects were removed from the home cage by enticing them into a small transport cage (30 × 32 × 34 cm) around 0700 h. Subjects in the small cage were immediately transferred to an unfamiliar room, where they remained until 1800 h (approximately 11 h) with limited olfactory and auditory contact and no visual contact with other marmosets.

Small Cage and Handling (SC+H). After collection of the first void sample of the day at 0700 h, subjects were caught using a net. The marmosets were removed from the net and held in gloved hands for 5 min. They were then immediately transferred

to a small transport cage (30 × 32 × 34 cm) for approximately 11 h as described in the conditions for SC. We assumed that housing a marmoset alone in a small cage for 11 h would constitute a mild stressor, and further assumed that net capture, followed by 5 min handling and 11 h single housing in a small cage would constitute a qualitatively more intense stressor.

Urine Collection

Urine samples were collected hourly, 0700 h to 1800 h inclusive, for all subjects during the control, SC, and SC+H conditions. The first void urine sample on the morning following a control and experimental day was also collected. Urine samples collected on control days, and the morning immediately following a control or experimental day were collected in a non–invasive manner from animals undisturbed in their home cage. All subjects had been trained to urinate in return for a desired food item. Urine was collected in hand–held aluminum pans. During the SC and SC+H conditions, when the subjects were housed in small transport cages, urine was collected opportunistically from large stainless steel pans under each cage. All urine samples were transferred to plastic vials, centrifuged at 700 rpm for 2 min and the supernatant transferred to a clean vial for storage at −20°C until assayed for cortisol.

Cortisol Enzyme Immunoassay (EIA)

Cortisol concentrations were measured in all urine samples using an enzyme immunoassay developed and validated for use in *C. kuhli*, modified from a cortisol EIA described previously (72) designed to measure urinary cortisol in samples from cotton–top tamarins. The antibody [R4866, raised against a steroid bovine albumin (BSA) in rabbit] and the steroid conjugate (horseradish peroxide) were diluted in EIA phosphate buffer to dilutions of 1:16,000 and 1:80,000 respectively. Standards were diluted in water and ranged from 1.95 to 1000pg (Sigma Chemical Company). Samples were diluted 1:6,400 in water. Serial dilutions of a urine pool collected from adult females in the first trimester of pregnancy and cortisol standards gave parallel displacement curves. Recovery of all standards (range 1.95–1000 pg) added to a low and medium quality control pool was 101% ± 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 in each sample were corrected for creatinine concentration, using a modified Jaffe end–point assay (68).

Statistical Analysis

Mean concentrations of urinary cortisol was computed for each animal, in each condition for the first void sample of the day (0600–0700 h), the morning period (0800–1200 h) and the afternoon period (1300–1800 h) and the data analyzed using a 3 factor mixed ANOVA. Sex of subject served as a between group factor (male vs. female) and time of day (first void sample of the day versus morning vs. afternoon) and stress condition (control vs. SC vs. SC+H) were within subject factors. In addition mean cortisol values for two h time blocks were calculated for each male and female and analyzed for each sex separately by a 2 factor ANOVA in each case. Factors were stress condition (control, SC and SC+H) and the 6 time blocks (0700–0800, 0900–1000, 1100–1200, 1300–1400, 1500–1600, 1700–1800 h). Comparisons between cortisol values in first–void urine samples collected the day after control and experimental treatments were made using a 2 factor ANOVA. Post–hoc analyses were conducted using Tukey HSD (34).

RESULTS

Urine Samples

We attempted to collect 12 samples (i.e. hourly samples 0700–1800 h) from each animal under each condition. For each condition, the following percent of the possible samples were collected: Control = 79%, SC = 91%, SC + H = 90%.

Circadian Pattern

All subjects exhibited a circadian pattern of hormone excretion under stress–free conditions (see Fig. 1). Analysis of the data by ANOVA revealed a significant main effect of time and sex on the diurnal pattern of cortisol excretion (Time, $F(9,84) = 3.29$, $p < 0.01$; sex, $F(1,84) = 9.43$, $p < 0.01$). First void samples reflected the accumulation of urine from the time of retreat the day previously (1800 h) until sample collection (0600 h), and the concentrations were low (<18 μg/mg Cr). Levels of urinary cortisol peaked around 1000–1100 h for both males and females. Following this peak in urinary cortisol, levels declined steadily in females to reach a nadir at 1800 h. In males, concentrations of urinary cortisol dropped dramatically in the early afternoon (1200–1300 h) and remained low for the remainder of the day.

Concentrations of urinary cortisol were higher in females than males at all time points throughout the day except 1700 (mean concentration of urinary cortisol ± SEM over the day; females 21.42 ± 1.7; males 14.24 ± 1.9 μg/mg Cr).

Response to Stressors

Exposing marmosets to the stressors SC and SC+H produced significant elevations in levels of urinary cortisol. Analysis of the

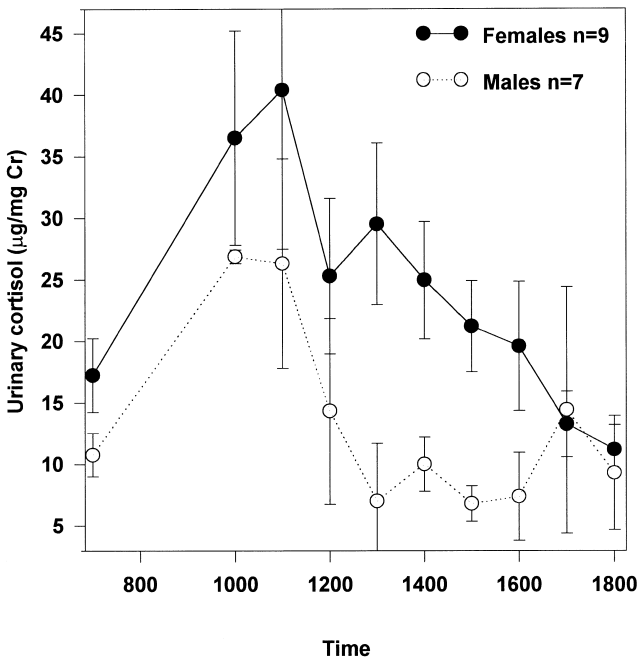

FIG. 1. Circadian rhythm of urinary cortisol secretion in males and females under stress-free conditions.

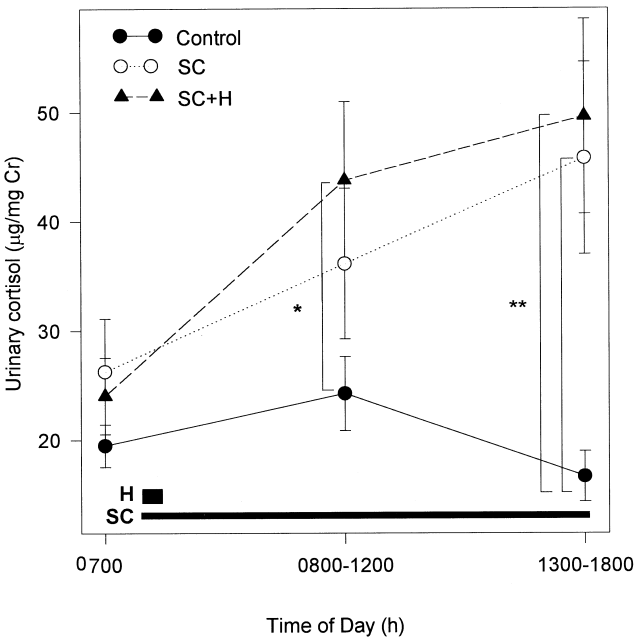

FIG. 2. Mean urinary cortisol values ± SEM in males and females for the first void sample of the day, the morning (0800–1200) and afternoon (1300–1800) periods under stress-free conditions (Control) and during small cage housing 0700–1800 (SC) and 5 min handling (H) followed by small cage housing. $*p < 0.05$; $**p < 0.01$.

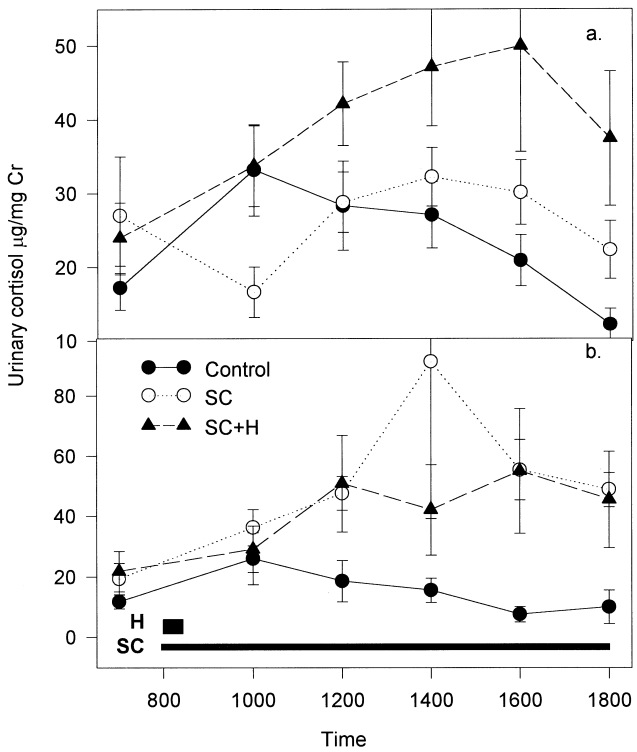

FIG. 3. Urinary cortisol in (a) females and (b) males under stress-free conditions (Control) and during small cage housing 0700–1800 (SC) and 5 min handling followed by small cage housing (SC+H).

data revealed a significant main effect of stress [$F(2,28) = 9.36$, $p < 0.001$] and a significant interaction between stress and time [$F(4,56) = 3.2$, $p < 0.025$; see Fig. 2]. Post hoc analysis revealed no significant differences between levels of urinary cortisol in the first void sample of the day for subjects across the three stress conditions. In morning samples collected on the day the stressor was administered, levels of cortisol were significantly higher under conditions of SC+H stress compared to control conditions ($p < 0.05$). There were no significant differences between urinary cortisol values in the morning under control conditions and exposure to SC. In the afternoon, levels of urinary cortisol following conditions of SC and SC+H were significantly higher than control values ($p < 0.01$ for both SC and SC+H).

There was also a significant interaction of sex by stress [$F(2,28) = 3.7$, $p < 0.05$] although post hoc pairwise comparisons revealed no significant differences. For males, exposure to a SC and SC+H stressors tended to produce higher levels of urinary cortisol and greater percent changes in cortisol compared to control conditions, than in females (see Table 2). Under stress–free control conditions, however, females had significantly higher urinary cortisol levels than males (see Fig. 1 and Table 2).

Analyzing cortisol values every two hour time block, for males and females separately for the three conditions, revealed sex differences in the adrenocortical response to stress. Data for females revealed a significant effect of stress and time [Stress, $F(2,119) = 12.23$, $p < 0.001$ and time, $F(5,119) = 2.67$, $p < 0.05$; see Fig. 3a]. Exposure to SC+H produced greater elevations in urinary cortisol than did exposure to the less stressful condition of SC alone (39.1 ± 3.5 vs. 26.4 ± 2.2 μg/mg Cr). Exposure to SC+H and SC significantly stimulated HPA activity compared to stress–free, control conditions (Control; 21.5 ± 1.8 μg/mg Cr; see Table 2). Data for males alone revealed a significant effect of stress, but not time [Stress, $F(2,83) = 6.81$, $p < 0.01$; Time, $F(5,83) = 1.5$, NS; see Fig. 3b]. HPA responses of

TABLE 2

ABSOLUTE AND RELATIVE CHANGES IN URINARY CORTISOL EXCRETION IN RESPONSE TO STRESSORS IN MALE AND FEMALE MARMOSETS

	Urinary Control			% Change from Baseline	
Sex	Control μg/mg Cr	SC[1] μg/mg Cr	SC + H[2] μg/mg Cr	SC % change	SC + H % change
Male	14.2 ± 1.9	49.5 ± 9	43.7 ± 6.2	412.9 ± 136	243.2 ± 78.8
Female	21.4 ± 1.7	26.4 ± 2.1	39.1 ± 3.5	40.4 ± 19.5	136.6 ± 41.9

[1] Small cage.
[2] Small cage and handling.

males, following exposure to SC and SC+H were similar, but in both cases, significantly greater than HPA activity under stress–free conditions (see Table 2).

There were no significant differences in cortisol concentrations in the first void sample of the day when the previous day had been a control day or days in which animals were exposed to SC or SC+H [$F(2,27) = 2.8$, NS. Post Control: 18.2 ± 3.1 μg/mg Cr; SC: 24.2 ± 3.3 μg/mg Cr; SC+H: 30.4 ± 4.7 μg/mg Cr]. Cortisol values in urine samples collected post control, SC and SC+H days were the same in males as in females [$F(1,37) = 1.2$, NS. Males: 20.9 ± 4.3; Females: 25.9 ± 2.6 μg/mg Cr].

DISCUSSION

The study has established that urinary cortisol can be used as a valid indicator of stress in marmosets. The circadian variation evident in plasma cortisol was also reflected in urinary cortisol values (e.g., 7,49,64). In addition, the adrenocortical response to SC+H, a relatively major stressor for animals in our colony, was discerned by elevations in urinary cortisol within a couple of hours of the stressor. Cortisol concentrations rose more rapidly, and remained elevated for significantly longer periods under conditions of the more severe stressor (SC+H) compared to the less severe stressor, SC alone. This study has thus demonstrated that our methods are sensitive enough to discern differential HPA responsiveness as a function of the severity of a stressor. A dose–response relationship between stressor intensity and urinary cortisol responsiveness has not been previously demonstrated in primates by measuring concentrations of urinary cortisol.

Concentrations of urinary cortisol for males and females across the day were in the same range as urinary cortisol values observed in other New World primate species [approximately 2–70 μg cortisol/mg Cr in the cotton–top tamarin (72) and 0.8–11.3 μg cortisol/mg Cr in goeldi's monkey *Callimico goeldii* (32)]. Baseline levels of urinary cortisol reported in the marmoset in the present study were, however, dramatically higher (i.e. 10–100 fold) than levels reported in Old World primates under stress free conditions e.g., humans (5–100 ng/mg Cr); mountain and lowland gorillas *G. gorilla* (20–70 and 5–130 ng/mg Cr respectively) and macaque *Macaca fascicularis* (0.3 μg/mg Cr) (18,13). Similarly, concentrations of plasma cortisol, as with other steroids in New World primates, were shown to be up to 100 fold higher than corresponding levels in Old World primates [reviewed in 39,10] (2,7,52).

The study documented a diurnal rhythm of urinary cortisol excretion in marmosets. Cortisol levels in the marmoset were highest in the early hours of the morning and declined to reach a nadir in the afternoon. Peak cortisol levels upon waking, and a trough before retiring to sleep, are observed in both diurnal and nocturnal species. High cortisol levels upon waking probably serve to mobilize energy stores in preparation for activity (60). The circadian pattern of urinary cortisol excretion observed in the marmoset in this study is similar to the circadian pattern of plasma cortisol secretion observed in other mammalian species such as humans (42), non–human primates [e.g. squirrel monkeys *Saimiri sciureus* (7); rhesus monkeys, *Macaca mulatta* (2,64); talapoin monkeys *Miopithecus talapoin* (49)] and rodents (19). Circadian variation in urinary cortisol excretion has so far only been established in hominoid primates, i.e. humans, lowland and mountain gorillas (18). The results from our study are valuable for future research since knowledge of cortisol excretion patterns under stress–free conditions is an essential prerequisite for (i) planning appropriate time frames in which to conduct stress manipulations [the adrenocortical response to a stressor is typically related to baseline cortisol values (3,7,33,58)] (ii) determining optimal sampling time in single–sample protocols [e.g., (69)] and (iii) predicting post–stress cortisol levels.

Baseline levels of urinary cortisol under stress–free conditions were higher in females compared to males, a result also reported for rodents (37). Studies examining sex differences in baseline cortisol values in humans and primates yield conflicting results. For example, in mouse lemurs, *Microcebus murinus*, but not squirrel monkeys, females had higher plasma cortisol levels than males under stress–free conditions (8,53). In humans, women had comparable baseline cortisol levels to males during the luteal phase of the cycle (63,66) but not the follicular phase, when levels were lower in females than males (74). Other studies have found no difference between cortisol levels in human males and females (25). Previous studies in humans and primates have established that plasma and urinary cortisol values are significantly altered by levels of circulating reproductive hormones—especially estrogens (9,22,57,64,72). In the present study, higher cortisol levels in females compared to males might have been associated with higher circulating levels of ovarian steroids. Further work on the potential for gonadal mediation of the stress response should investigate the HPA responsiveness to a variety of physical and psychological stressors in reproductively active versus reproductively suppressed marmosets.

Exposure to stressors produced significant increases in levels of urinary cortisol in both males and females. In our case, net capture, isolation in a small cage, and even short–term manual restraint are atypical procedures and it may be that HPA responsiveness would decrease with repeated capture and handling, as the animals adapt. Primates have been shown to adapt to stressful conditions so that stimuli that once produced elevated cortisol levels cease to do so following a period of familiarization and adaptation [(5,13) but see (8)]. The stressors used in the present study might have no effect on HPA activity in primates that experience frequent capture and restraint for regular blood sampling.

There was no difference in the concentration of the first void sample of the day collected prior to any experimental manipulation. The levels of cortisol in the first void urine sample was a reflection of cortisol that had accumulated from early evening the previous day, until sample collection. Since cortisol levels did not vary prior to stress manipulation across all conditions, any increases in cortisol levels the day of the stressor were produced by the stressor and not extraneous variables operating in the 12 h period prior to stress administration. SC+H but not SC alone produced significant elevations in urinary cortisol over the morning period compared to control conditions. This is the first study, to our knowledge, to examine the effect of short term manual restraint on HPA activity in primates. Several studies have, however, examined HPA activity in primates in response to a combination of capture combined with restraint, anesthesia and venipuncture with each study producing very different results [e.g., see 32,58,72].

In our subjects, urinary cortisol rose rapidly in response to stress since a cortisol response to SC+H was apparent before 1200 h, i.e. within 4 h of the stressor. Cortisol has a fast clearance rate, however, since although cortisol levels were significantly elevated in subjects at 1800 h following a stressor, the first void sample of the next day was not significantly higher than baseline values. The cortisol response to a stressor has been shown in some (67) but not all studies (3), to differ depending on the point during the circadian rhythm that the stressor was experienced. This might be an important point to consider when discussing HPA response to stressors. In the present study, all sub-

jects experienced the onset of the stressor at the same time (i.e. around 0700).

Males exhibited a greater relative increase (i.e. percentage change) in levels of urinary cortisol following exposure to the stressors compared to females. Baseline urinary cortisol values in males were significantly lower than baseline cortisol levels in females. Previous studies with humans (67); non–human primates (7,48,58) and rodents (3) have established that the magnitude of the stress response (i.e. percentage change in cortisol levels from basal values in response to a stressor), is typically related to initial baseline cortisol values, with the greatest stress response being manifested when baseline values were low [see 8]. The results from our study that males, with initially the lowest baseline cortisol values, exhibited the greatest percentage change in urinary cortisol post–stress, is in agreement with these latter studies (3,7,48,58,67). Low baseline adrenocortical output and a large and rapid adrenocortical response to a stressor is observed in dominant individuals of several Old World primate species, and spares the dominant individuals from the detrimental consequences of a prolonged stress response [(6,35,55,58) but see (57,72)]. Callitrichids exhibit strong, well defined intra–sexual dominance hierarchies [e.g., (1)]. Inter–sexual patterns of dominance are less clear, however, meaning low baseline cortisol values and large adrenocortical responses to stress in males in our study can probably not be explained in terms of dominance effects.

Exposing animals to a relatively minor stress (housing in small transport cage) produced significantly raised levels of urinary cortisol in the afternoon period compared to control values. In our study, the conditions of minor stress (housing in a small transport cage) was composed of several social and physical factors that have been shown previously to stimulate HPA activity in primates. First, animals were confined to a small area with no enrichment. Previous studies examining the effect of cage size on HPA activity in primates have either shown a significant effect (5) or no effect (12,13,14,15,45,46) of cage size. Second, marmosets in our study were physically and visually separated from familiar group members, a potent source of stress in primates (44,52). Third, separated marmosets were placed at floor level, which has been shown to produce elevations in cortisol levels [(54,71), but see (13)]. Four, animals were housed in an unfamiliar colony room. Unfamiliar surroundings stimulate HPA activity in several primate species (13,16,30). Any combination of the above social and physical conditions might have produced elevated cortisol under the stress conditions.

The study raises important husbandry issues, since it established that (i) holding a monkey for as little as 5 min (as occurs during routine veterinary examinations) and (ii) housing animals in temporary isolation in a relatively small cage (as might occur during transportation or when an animal is sick and requiring veterinary attention) constitute potent stimulators of HPA activity. The detrimental effects of prolonged periods of HPA activity such as suppressed immune function, cell death and suppressed reproduction, are well established (6,55,60). It is important to test in the future, therefore, the long–term impact of relatively minor husbandry manipulations on HPA functioning.

The adrenocortical response to what we assumed to be a more major stressor (SC+H) was more rapid and prolonged than the adrenocortical response to a relatively minor stress (SC). A more rapid and extended HPA axis response to major compared to a minor stressor suggests that the marmoset HPA axis displays differential responsiveness as a function of the severity of the stressor and that this is reflected in levels of urinary cortisol. A dose response relationship has been demonstrated previously in primates between plasma cortisol concentrations and social stressors in the context of mother–infant separation (70). In rats a dose–response has been demonstrated between severity of a physical stressor (e.g., hypotension) and plasma adrenocorticotropic hormone (20). Since the physiological components of a stress response can have detrimental consequences on the body if prolonged over a period of time, it is important that concentrations of circulating cortisol are optimal to ensure there is enough cortisol to implement the physiological processes necessary to survive the stressor while at the same time imposing minimal negative effects on the body.

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