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# Close Proximity of the Heterosexual Partner Reduces the Physiological and Behavioral Consequences of Novel-Cage Housing in Black Tufted-Ear Marmosets (*Callithrix kuhli*)

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**The present studies assessed the extent to which heterosexual pairmates could buffer marmosets (Wied's black tufted-ear marmoset, *Callithrix kuhli)* against stress. Six male and six female marmosets from established groups were exposed to two experimental manipulations together with a control condition. Each condition lasted a total of 4 days. For the two experimental conditions, animals were removed from the family group and housed in a novel cage for 48 h in either the presence or the absence of the heterosexual pairmate. During the 48-h novel-cage housing period and for 48 h upon reunion of the subjects with the family group, concentrations of urinary cortisol were measured in the first void sample of the day and behavioral observations were conducted. When animals were housed alone in a novel cage they exhibited significant elevations in levels of urinary cortisol after 24 and 48 h of novel-cage exposure. In contrast, when marmosets were housed in the novel cage in the presence of the pairmate, levels of urinary cortisol did not change across the 4-day period. The presence of the social partner also reduced the behavioral manifestations of exposure to novelty. Upon reunion with the family group, animals that had been housed in the novel cage alone spent significantly more time in close proximity to the pairmate than animals that had been housed with the partner. A second experiment was conducted to determine the effect that separation from the pairmate, only (independent of any effects of novelty), had on levels of cortisol. Concentrations of urinary cortisol were measured in subjects housed in the familiar home cage, but in the absence of the pairmate, over a 48-h period and compared to concentrations of excreted cortisol immediately prior to separation. Separation from the pairmate did not elevate cortisol levels when the subject was housed in the home cage, suggesting that elevated cortisol levels in animals housed alone in the novel cage were in response to novelty exposure rather than to separation from the pairmate. Since the physical presence of the heterosexual partner reduced the physiological and behavioral effects of novel-cage housing, social attachments might function as homeostatic regulators of HPA function in marmosets.**



***Key Words:*** **marmosets; callitrichids; urinary cortisol; social support; separation; novelty; stress.**

The presence of significant social partners can have salutary effects on the physiological and behavioral consequences of exposure to a physical or psychological stressor. This has been conceptualized as a "social buffering" or "social support" effect, the benefits of which have been described in two separate models (Cohen and Wills, 1985). A main effect model proposes that integration into a social network provides an individual with stability, predictability, and a sense of positive self-worth. Together, these positive experiences create general well-being, good health and an increased ability to cope with stress should it occur (e.g., Kawachi, Colditz, Asherio, Rimm, Giovannucci, Stampfer, and Willett, 1996; Stevens, 1997; Cheng, 1997). The main effect model proposes that the beneficial effects of social support operate continually on an individual irrespective of whether or not stress is

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being experienced. The buffering model, on the other hand, proposes that the beneficial effects of social factors on stress occur directly at the time of the stressor to either modulate the perceived intensity of the stress stimuli or to downregulate the magnitude of the physiological response to these stimuli (Levine, 1993). The present study was designed to assess the latter model, the buffering model of social support, in a social primate Wied's black tufted-ear marmoset, *Callithrix kuhli.* The buffering model of social support is supported by studies conducted on both human (e.g., Lepore, Allen, and Evans, 1993; Kirschbaum, Klauer, Sigrun-Heide, and Hellhammer, 1995; Short and Johnston, 1997) and nonhuman primate populations; the majority of studies in the latter case being conducted in the context of mother-infant relations. Infants separated from their mother exhibit dramatic behavioral indices of distress (Levine, Wiener, and Coe, 1993) in addition to adrenocortical activation (Coe, Mendoza, Smotherman, and Levine, 1978; Mendoza, Smotherman, Miner, Kaplan, and Levine, 1978; Laudenslager, Boccia, Berger, Gennaro-Ruggles, McFerren, and Reite, 1995) and suppressed immunity (Friedman, Coe, and Ershler, 1991; Coe and Erickson, 1997; see reviews in Mineka and Suomi, 1978; Coe, Wiener, and Levine, 1983). The physiological and behavioral consequences of separation-induced stress in the infant are dampened, however, if additional social partners are present during the separation period (e.g., Coe, Rosenberg, Fischer, and Levine, 1987; Hennessy, 1984).

Demonstrations of social buffering in primates outside of the mother-infant dyad are limited (but see Stanton, Patterson, and Levine, 1985). Furthermore, examples of social buffering involving relationships other than the mother-infant pair are often confounded by the effects of separation (e.g., Gust, Gordon, Brodie, and McClure, 1994; Gunnar, Gonzalez, and Levine, 1980). For example, in several species of primates, exposure to novelty in the absence of a social partner produces physiological signs of stress such as increased adrenocortical activity (e.g., Hennessy, Mendoza, Mason, and Moberg, 1995) and suppressed immune function (e.g., Gust *et al.*, 1994). The presence of a significant social partner during novelty exposure can, in some species, reduce the physiological or behavioral manifestations of the manipulation (Hennessy *et al.*, 1995). However, studies addressing social buffering often lack the controls needed to tease apart the independent effects of separation from social partners versus exposure to novelty on the physiological manifestations produced by the manipulation (e.g., Gust *et al.*, 1994). Consequently, these studies are unable to determine the extent to which reduction in cortisol levels observed during novelty exposure in the presence of a partner was caused by (i) reunion with a partner or (ii) social buffering against the effects of environmental novelty.

The way in which social factors affect the stress response in a species varies dramatically, depending on the nature of social attachments and interaction patterns exhibited by the species. Within the primate order, there is considerable interspecific variation in group size, mating system, offspring-rearing strategies, and, most importantly for this study, in the nature of significant social attachment and interaction patterns (Smuts, Cheney, Seyfarth, Wrangham, and Struhsaker, 1987). As a consequence of these profound species differences, there is considerable variation in the ways in which social factors modulate the stress response. For instance, the nature of social relationships differs dramatically in squirrel monkeys (*Saimiri*) and titi monkeys (*Callicebus*). Squirrel monkeys live in large, mixed-sex social groups, in which affiliative interactions occur predominantly between same-sex individuals (Baldwin, 1985). In contrast, *Callicebus* live in small territorial groups with an adult heterosexual pair as the core (Mason, 1966). *Callicebus* and *Saimiri* exhibit contrasting behavioral and physiological responsiveness during short-term separations from heterosexual partners. Separation has more dramatic effects in *Callicebus,* and during separation animals exhibit increased levels of distress and agitation, increased heart rate, and elevated plasma concentrations of cortisol, while *Saimiri* is neither behaviorally nor physiologically responsive to separation from heterosexual partners (Cubicciotti and Mason, 1975; Mendoza and Mason, 1986). Furthermore, exposure to novel environments produces elevated cortisol responses in both species, but the presence of a heterosexual partner reduces this response in titi monkeys, but not in squirrel monkeys (Hennessy *et al.*, 1995).

In the callitrichid primates (marmosets and tamarins), one of the most significant social attachments is the strong, long-term sociosexual pair bond that exists between the breeding male and female in a social group (Evans, 1983; Anzenberger, 1993; Schaffner, Shepard, Santos, and French, 1995). The pair bond is characterized by high rates of affiliative behavior such as grooming, huddling, and close spatial proximity between the breeding male and female (Vogt, 1978; Schaffner *et al.*, 1995). We designed two experiments to test the buffering model of social support in heterosexual pairs of Wied's black tufted-ear marmosets (*C.*

**TABLE 1**
Details of the Study Groups

| Group | Years of pair formation | Brother of breeding male | Number of offspring present |
|---|---|---|---|
| Reb | 4.4 | Absent | 4 |
| Bon | 1.5 | Present | 2 |
| Bai | 1.2 | Present | 5 |
| Ang | 5.0 | Absent | 2 |
| Wir | 1.5 | Present | 2 |
| Cor | 4.0 | Absent | 1 |

*Note.* The table indicates the number of years the breeding pair had resided together and the number of offspring in the group at the start of the study. Information is also presented on whether or not the brother of the breeding male was present throughout the study.

*kuhli*). Animals were exposed to novelty in either the absence or the presence of social partners. We made the following predictions. First we predicted that housing black tufted-ear marmosets in a novel cage would be stressful (see Hennessy, 1984; Hennessy *et al.,* 1995). Second, in light of the strong heterosexual pair bond between the breeding male and female, we predicted that the physical presence of the heterosexual pairmate at the time of housing in a novel cage would reduce the magnitude of the behavioral and endocrine response to this manipulation. A second experiment, in which urinary cortisol was monitored in animals that remained in their home cage, but in the absence of their pairmate, served as a control to assess the effect of separation from the pairmate only (see Hennessy, 1997).

## GENERAL METHODS

### Subjects

The study involved six family groups of black tufted-ear marmosets (*C. kuhli*) housed at the University of Nebraska at Omaha Callitrichid Research Center. Each family group was composed of a breeding male and female together with their descendant offspring (see Table 1 for details of family composition). In three family groups, the brother of the breeding male was also present. The breeding male and female of the group served as the focal subjects in each manipulation. The groups were only involved in experimental manipulations when the breeding female was in the first trimester of pregnancy (see French, Brewer, Schaffner, Schalley, Hightower-Merritt, Smith, and Bell, 1996). Females were only included in the study during the first trimester of pregnancy to control for changes in cortisol levels associated with gestation. Cortisol concentrations in this species remain at baseline (nonpregnant) levels during the first trimester of pregnancy and increase throughout gestation to reach significantly elevated levels by the third trimester (Smith and French, 1997a). The breeding pair had resided together for a minimum of 1.0 year. Animals under all conditions were housed in large cages measuring 1.6 × 0.9 × 2.4 m, equipped with natural branches, a platform, and a nest tube. No more than two family groups were housed in a single colony room. Marmoset groups within a colony room had olfactory and auditory contact but no visual contact. Natural lighting was supplemented with fluorescent lighting (12 h light:12 h dark) with light onset at 0600 h. Further husbandry details are provided by Schaffner *et al.,* (1995).

### Sample Collection and Determination of Urinary Cortisol Concentrations

Under all conditions, the first void urine sample was collected each day in a noninvasive, stress-free manner (Smith and French, 1997a,b). All animals in our colony are trained to urinate into small, hand-held pans in return for a desired food item. Urine samples (typically 0.2–1.0 ml) were transferred to plastic vials and centrifuged at 700 rpm for 2 min, and the supernatant was transferred to clean vials and stored at −20°C until assayed.

Concentrations of urinary cortisol were determined in each sample using an enzyme-immunoassay validated for use in *C. kuhli* and described previously by Smith and French (1997b). We have shown previously in *C. kuhli* that levels of cortisol excreted in the urine accurately reflect levels of cortisol circulating in the plasma both under control conditions and following administration of dexamethasone, a synthetic glucocorticoid that suppresses release of adrenocorticotrophic hormone from the pituitary and subsequent release of cortisol from the adrenals (Smith and French, 1997c). The intraassay coefficients of variation for high and low concentration pools were 4.4 and 4.3%, respectively ($n = 31$). Interassay coefficients of variation for high and low concentration pools were 16.1 and 13.8%, respectively ($n = 31$). To control for variations in urine dilution, hormone concentrations were corrected for the creatinine concentration of each sample. Creatinine concentration was measured by a modified Jaffé end-point assay (Tietz, 1976). Samples were not included in the analysis if the creatinine

value was less than 0.1 mg/Cr, i.e., below the minimum sensitivity level of the assay.

### Behavior Observations

Behavior observations were only conducted on subjects under conditions in Experiment 1. Focal animal behavior observations (20 min in length) were conducted on all subjects between 0800 and 0900 h, at least 30 min following the morning feeding. Behaviors were recorded by an observer, familiar to the animal, sitting quietly in the colony room, approximately 2 m from the cage using the Observer 3.0 computerized behavioral recording program loaded onto a 486 laptop computer. The number of 20-s instantaneous samples per 20 min that animals were locomoting was recorded to provide an indication of the animal's levels of distress and agitation. In addition, we recorded the number of 20-s instantaneous samples that the breeding pair were in (i) close proximity (i.e., within 10 cm) to one another and (ii) engaging in social behavior (i.e., grooming, copulation, or attempted copulation). All occurrences of twitter vocalizations and scent marking (circumgenital marks and chest-rubs combined) were also recorded (see Schaffner *et al.*, 1995; French, Schaffner, Shepherd, and, Miller, 1995; for further details of behavioral categories and observational protocols).

## EXPERIMENT 1

### Experimental Design

Using a repeated measures design, all subjects (i.e., six breeding males and six breeding females) experienced two experimental manipulations and one control condition. For the two experimental manipulations, around 0700 h on Day 1, following collection of the first void urine sample of the day, subjects were removed from the home cage in a noninvasive manner by enticing them into a small transport cage attached to the side of the home cage. Immediately thereafter the subject was transferred into a novel, clean cage ($2.1 \times 1 \times 0.75$ m) positioned 0.75 m from the home cage. Subjects in the novel cage had full visual, olfactory, and auditory contact with the home cage and family members therein, but no physical contact. Subjects had no visual contact, but were in auditory and limited olfactory contact with a second family group in the room, i.e., as happens under normal conditions when the subject is in the home cage. Subjects were housed in the novel cage in either the absence (*Novel-absent*) or the presence (*Novel-present*) of the pairmate. A single Novel-present manipulation therefore counted as the Novel-present condition for the female partner and male partner at the same time. The pairmates were allowed to interact freely under the Novel-present condition. The two experimental manipulations, Novel-present and Novel-absent, composed 4 consecutive days: a novel-housing phase lasting 2 days and a reunion phase lasting 2 days (see Fig. 1). Under the *Control* condition, subjects were housed undisturbed in the home cage in the presence of all social partners and their behavior and endocrine status monitored for 4 consecutive days. The order in which subjects experienced each manipulation varied across individuals.

### Statistical Analysis

Levels of urinary cortisol were assessed in males and females under the Novel-absent, Novel-present, and Control conditions using a mixed three-factor ANOVA (Sex $\times$ Condition $\times$ Day of manipulation). Changes in behavior scores for locomotion, twitter, and scent mark across the experimental conditions were assessed using a mixed design, four-factor ANOVA (Sex $\times$ Condition $\times$ Phase $\times$ Day). The factor "phase" in the analysis represented the separation and reunion phases since we expected levels of behavior across any one condition to be different in these two periods. Under the Novel-absent condition, any association between behavior scores (locomotory behavior and twitter vocalizations) and concentrations of urinary cortisol was assessed using Pearsons correlation. In all cases, a correlation was assessed between behavior scores observed on a day and concentrations of urinary cortisol measured in the first void sample of the following morning. Concentrations of cortisol excreted in the first void sample of the day probably reflect levels of urinary cortisol that accumulated in the urine from time of retreat the following evening to the time of morning urination.

The effect of experimental manipulation on the behavior of pairs upon reunion of the partner(s), i.e., amount of time the pair spent in close spatial proximity and engaging in social behavior, was assessed using mixed three-factor ANOVAs (Sex $\times$ Condition $\times$ Day). When a significant interaction was found, data were analyzed separately using one-way ANOVAs. Post hoc analyzes were made in all cases using Tukey HSD.

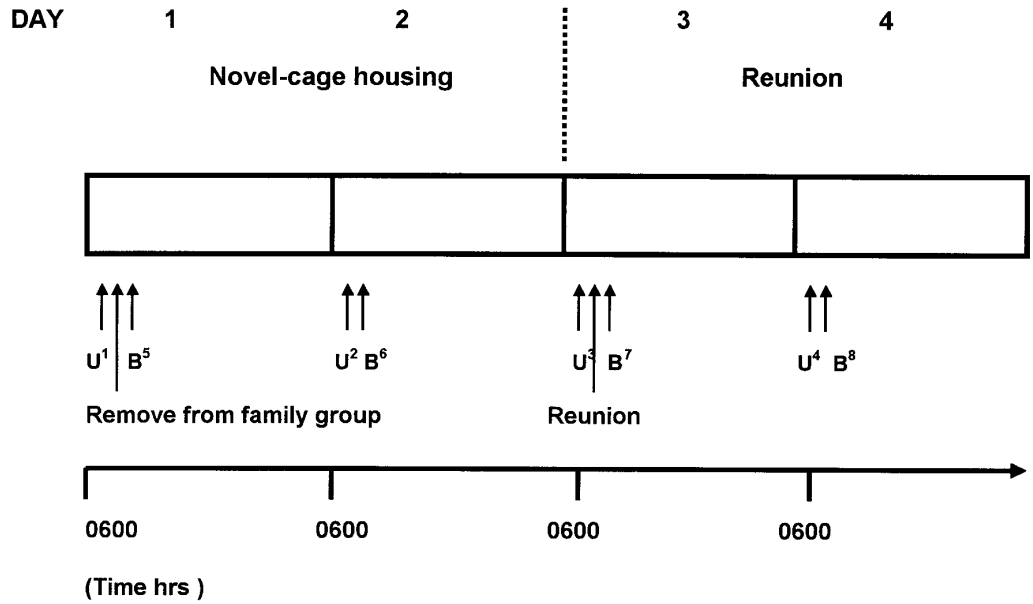


**FIG. 1.** Experimental design showing the 2-day novel-housing phase and 2-day reunion phase. Subjects were housed in a novel cage in both the absence and the presence of the opposite-sex pairmate. U, urine sample collection; B, behavior observation. Urine samples were collected immediately prior to the manipulation ([1]Pre-sep), 24 h following novel-cage housing ([2]24-Novel), 48 h following novel-cage housing ([3]48-Novel), and 24 h following reunion with the family group in the familiar home cage ([4]24-Reunion). Behavior observations were conducted immediately following novel-cage housing ([5]0.5-Novel), 24 h following novel-cage housing ([6]24-Novel), immediately following reunion ([7]0.5-Reunion), and 24 h following reunion ([8]24-Reunion).

## EXPERIMENT 2

### *Experimental Design*

Experiment 2 assessed the effect of separation from the pairmate only, independent of any effects of novel-cage housing, on concentrations of urinary cortisol. Levels of urinary cortisol were assessed in the six males and six females as they resided for 48 h in the familiar home cage in the absence of the pairmate but with all other social partners. The pairmate was housed in a separate cage positioned 0.75 m from the home cage in full visual, olfactory, auditory but not physical contact. Urine samples were collected immediately prior to removal of the pairmate and 24 and 48 h later and levels of excreted cortisol measured as described for Experiment 1.

### *Statistical Analyses*

Levels of urinary cortisol were analyzed using a two-factor mixed ANOVA (Sex × Condition) comparing cortisol concentrations preseparation (i.e., immediately prior to separation) with cortisol values during separation. For each subject, cortisol concentrations during the separation phase were computed as the mean concentration of excreted cortisol 24 and 48 h following separation.

## RESULTS: EXPERIMENT 1

### *Endocrine Response to Experimental Conditions*

The experimental manipulations had significant effects on levels of urinary cortisol and behavior patterns. Analysis of urinary cortisol concentrations revealed a significant interaction between experimental condition and day [$F$ (3, 30) = 3.40, $P < 0.05$; Fig. 2]. Under the Novel-absent condition, levels of urinary cortisol changed significantly over the 4-day manipulation period [$F$ (3, 33) = 11.72, $P < 0.01$]. Subjects exhibited significantly increased levels of urinary cortisol after 24 and 48 h ($P < 0.05$) exposure to novel-cage housing compared to baseline cortisol values. Cortisol levels returned to baseline concentrations 24 h following reunion in the home cage. In contrast, cortisol levels under the Novel-present and Control conditions did not vary significantly over the 4-day manipulation period [$F$ (3, 33) = 1.97 and 0.83, respectively, NS]. Concentrations of urinary cortisol did not vary between males and females.

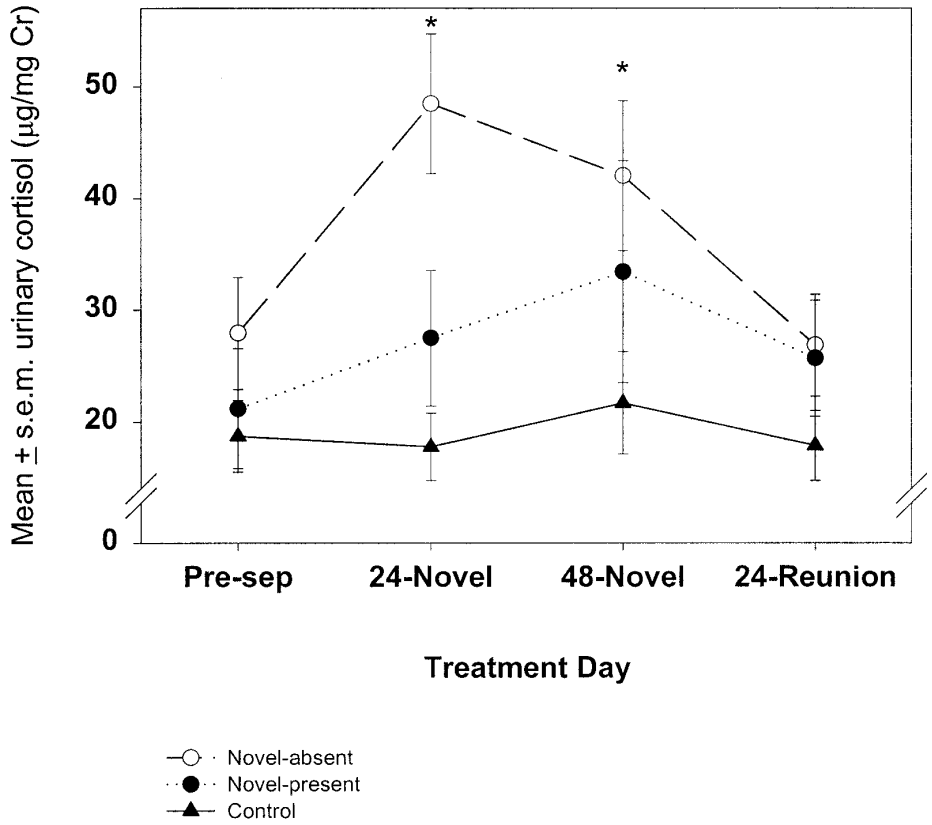


**FIG. 2.** Concentrations of urinary cortisol for animals housed in the novel cage in the absence (Novel-absent) and presence (Novel-present) of the opposite-sex pairmate and under control conditions (Control). Levels of urinary cortisol are shown for samples collected immediately prior to the manipulation (Pre-sep), 24 h (24-Novel), and 48 h (48-Novel) following novel-cage housing and 24 h following reunion with the family group in the familiar home cage (24-Reunion). Levels of urinary cortisol were significantly elevated in animals housed under the Novel-absent condition 24 and 48 h following novel-cage housing, $*P < 0.05$.

### *Behavioral Response to Experimental Manipulations*

Housing marmosets in a novel cage in the absence of the pairmate produced significant changes in behavior patterns indicative of distress and agitation compared to control conditions. The behavioral manifestations produced by the manipulation were eliminated, however, if animals were housed in the novel cage with the pairmate (i.e., Novel-present). Levels of locomotory behavior for the 4-day manipulation period combined varied significantly across experimental conditions [$F$ (2, 20) = 3.74, $P < 0.05$, Fig. 3a]. Marmosets exposed to the Novel-absent condition spent significantly more intervals locomoting (10.15 ± 1.19) during the 4-day period than they did under the Control condition (6.31 ± 0.69; $P < 0.05$). In contrast, animals under the Novel-present condition exhibited rates of locomotory behavior (7.67 ± 0.91) over this period that did not vary from levels observed under the Control conditions. Under the Novel-absent condition, levels of locomotion during the first day of novel-cage housing were positively correlated with concentrations of urinary cortisol in the first void sample collected the following morning ($r = 0.66$, $P < 0.02$). There was no significant correlation, however, between locomotory behavior during the second day of novel-cage housing and concentrations of urinary cortisol ($r = -0.43$, NS) or the first day of reunion and concentrations of urinary cortisol ($r = -0.06$, NS).

Analysis of the number of twitter vocalizations produced by subjects during the experiment revealed a significant interaction between condition and day [$F$ (2, 20) = 8.64, $P < 0.01$; Fig. 3b]. Further analysis of the data using one-way ANOVAs showed that the number of twitters produced on Day 1 varied significantly across the three conditions [$F$ (2, 22) = 6.14, $P < 0.01$]. Marmosets produced more twitters on Day 1 of the manipulation under the Novel-absent condition than

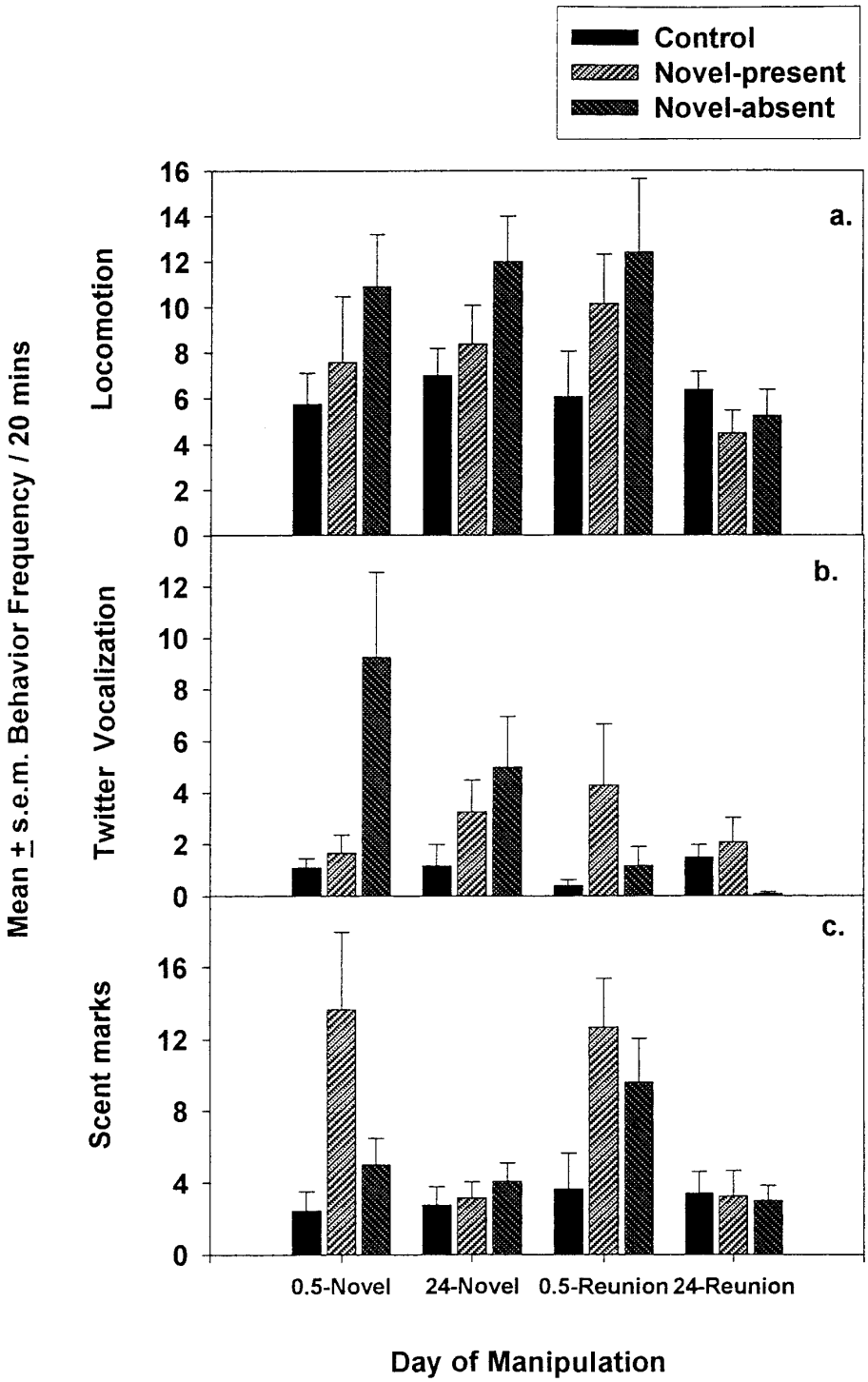


**FIG. 3.** Scores are shown for behaviors exhibited by animals housed in the novel cage in the absence (Novel-absent) and presence (Novel-present) of the heterosexual pairmate and under control conditions (Control). Data are presented across the 4-day manipulation period immediately following novel-cage housing (0.5-Novel), 24 h following novel-cage housing (24-Novel), immediately following reunion (0.5-Reunion), and 24 h following reunion (24-Reunion). Results are shown for mean number ± SEM of 20-s instantaneous scans per 20 min that animals were observed (a) to be locomoting. (b) Mean number ± SEM twitters produced and (c) mean ± SEM scent marking frequency. See text for details of significant differences across manipulations phases, days of manipulation, and condition.

they did on Day 1 under either the Novel-present or the Control conditions ($P < 0.05$). There was no correlation between twitter frequency and cortisol concentrations on any day during the Novel-absent manipulation (cortisol concentrations and twitter frequency: first day of novel-cage housing, $r = -0.18$, NS; second day of novel-cage housing, $r = -0.43$, NS; and first day of reunion, $r = 0.24$, NS).

The patterning of scent marking behavior exhibited by males and females was modified by the experimental condition [$F$ (2, 20) = 6.17, $P < 0.01$, Fig. 3c] and day of the manipulation [$F$ (1, 10) = 17.68, $P < 0.01$]. A significant interaction was found for scent marking behavior between condition and day [$F$ (2, 20) = 8.64, $P < 0.01$]. Animals under the Novel-present condition demonstrated significantly elevated levels of scent marking on the first days of the separation and reunion phases than on the second days of these phases ($P < 0.01$). Marmosets under the Novel-absent and Control conditions, however, demonstrated comparable rates of scent marking on both the first and the second days of the separation and reunion phases. Scent marking scores did not vary between males and females.

The behavior of marmosets when they were reunited with the family group differed depending on whether or not the pairmate had been present during novel-cage housing. The presence or absence of the pairmate significantly affected the amount of time pairmates spent in close proximity during the reunion phase [$F$ (2, 20) = 5.80, $P < 0.025$; Fig. 4a]. Marmosets spent significantly more time in close proximity following novel-cage housing in the absence of a pairmate compared to the Novel-present and Control conditions ($P < 0.05$). In contrast, there was no difference between the amount of time animals spent in close proximity under the Novel-present and Control conditions. The amount of time that the breeding pair engaged in social behavior upon reunion was not affected by the absence versus presence of a social partner during housing in the novel cage [$F$ (2, 20) = 2.44, NS; Fig. 4b].

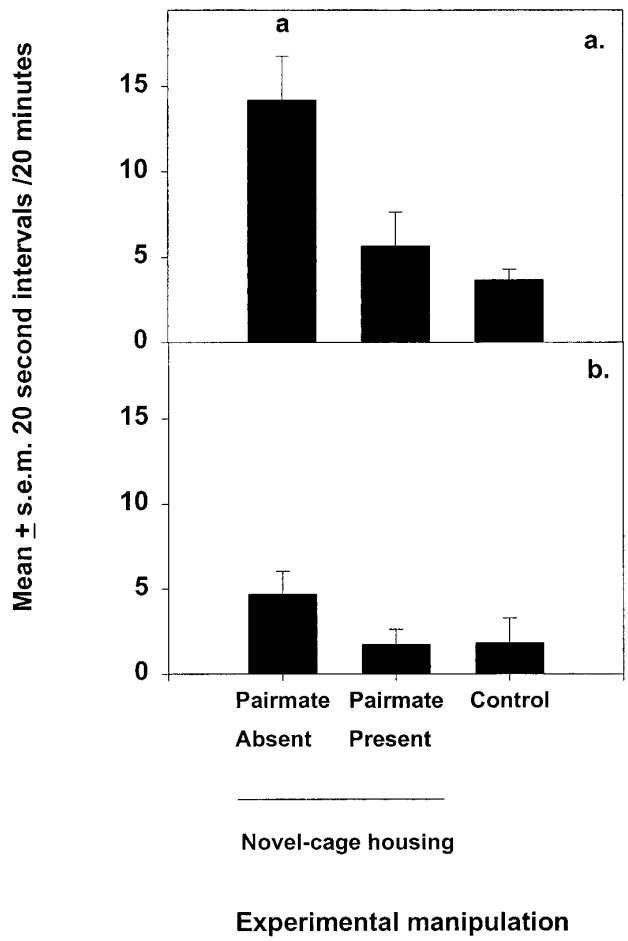


**FIG. 4.** Mean number ± SEM of 20-s instantaneous scans per 20 min that the breeding pair were observed to be (a) in close proximity to one another (i.e., within 10 cm) and (b) engaging in social behavior, for 2 days under control conditions (Control) and upon reunion following novel-cage housing in the absence (Novel-absent) and presence (Novel-present) of the pairmate. The behavior scores shown are mean behavior score for observations conducted immediately upon reunion and 24 h following reunion. (a) $P < 0.05$ vs Control.

## RESULTS: EXPERIMENT 2

When marmosets were housed in the familiar home cage but in the absence of the pairmate, they did not exhibit elevated levels of urinary cortisol compared to baseline conditions [mean urinary cortisol concentrations ± SEM, preseparation, 15.15 ± 2.29; and separation, 15.70 ± 2.47; $F$ (1, 10) = 0.15, NS]. There were no sex differences in concentrations of excreted cortisol under these conditions.

## DISCUSSION

The experiments suggested that environmental novelty is a potent stimulant of HPA activity in marmosets: When marmosets were housed alone in a novel cage, they exhibited increased levels of urinary cortisol together with behavioral changes indicative of distress and agitation. Since separation from the pairmate

only, in the absence of any effects of novelty, did not cause increased indices of distress (experiment 2), elevated cortisol concentrations during novel-cage housing were probably caused by exposure to novelty. The presence of the heterosexual pairmate during novel-cage housing dampened the HPA response to the novel environment and eliminated the associated behavioral manifestations. The present study therefore demonstrates a social buffering effect in marmosets and, unlike several previous studies assessing social buffering (e.g., Gust *et al.*, 1994; Wiener, Bayart, Faull, and Levine, 1990; Gunnar *et al.*, 1980), has the advantage of eliminating the confound of separation from social partners as a cause of elevated cortisol levels during stressor exposure (see Hennessy, 1997).

One example of social buffering in primates that does control for separation effects on HPA activity during stressor exposure is provided by Hennessey *et al.* (1995). In titi monkeys, the strongest social relationship in the group develops between adult males and females (Mason, 1966). As was demonstrated in the present study, HPA activation in response to novel housing conditions in titi monkeys was reduced by the presence of the opposite sex pairmate (Hennessy *et al.*, 1995). In contrast, in the squirrel monkey, strong social attachments occur between same-sex as opposed to opposite—sex individuals (Baldwin, 1985) and opposite-sex animals are able to buffer conspecifics against the stress induced by novelty (Hennessy *et al.*, 1995). In callitrichid primates, the relationship between the breeding male and female constitutes the strongest social tie within the group (Hightower and French, unpublished results; Vogt, 1978; Evans, 1983). The present study has shown that the presence of the heterosexual social partner is able to modulate the physiological consequences of exposure to a stressor. Previous studies have highlighted substantial individual variation in the quality of this relationship in marmosets (Schaffner *et al.*, 1995). It would be interesting in future studies to ascertain the extent to which subtle variations in the quality of relationships between pairmates influence the strength of the buffering properties (see Gust, Gordon, Hambright, and Wilson, 1993a).

In the current study, the elevated levels of cortisol in animals that had been housed alone in the novel cage returned to baseline values within 24 h of reunion with family members in the familiar cage. We suggest three possible explanations for the observed decline in cortisol concentrations. First, simply terminating novelty exposure may have reduced HPA activity and, subsequently, cortisol concentrations in marmosets. Second, marmosets may have habituated to the novel environment after 2 days in the novel cage resulting in a return of cortisol values to baseline concentrations. Results from previous studies, however, suggest that the decrease in cortisol values observed upon reunion was not caused by habituation of the HPA axis since black tufted-ear marmosets removed from the family group and housed in a novel cage for a prolonged period of time (e.g., 1 month) exhibit consistently and significantly elevated levels of urinary cortisol throughout the separation period (Smith and French, 1997a).

A third explanation for the reduction in cortisol concentrations in reunited animals is that close spatial proximity with social partners upon reunion may have served to reduce HPA activity. Marmosets spent more time in close proximity to the pairmate upon reunion after they had been housed in the novel cage alone, relative to being housed in the novel cage with the pairmate. Close spatial proximity to the pairmate might reduce HPA activity in marmosets. Previous work in our laboratory utilizing a short-term separation - reunion paradigm has suggested that separated marmosets may use high levels of affiliative social behavior and close proximity upon reunion as a mechanism to dampen stress, whereas markers of stress remain high in lion tamarins, who engage in low levels of social behavior and proximity upon reunion with a separated partner (Shepherd and French, in press). Similar results are observed in the context of other relationships in a variety of species. For example, there is a positive correlation between the amount of time juvenile sooty mangabeys spent in close proximity with and huddling with the mother and the recovery in the number of total T cells after a prolonged separation (Gust, Gordon, Brodie, and McClure, 1993b). Similarly, in an established group of female rhesus macaques, higher rates of affiliative social behavior, such as huddling and grooming, were associated with *reduced* plasma cortisol (Gust *et al.*, 1993a). Together, these data suggest that high levels of affiliative behavior and close spatial proximity may be potential social strategies to reduce HPA function. Affiliative interactions with social partners may be as important a contributor to reduced HPA activity as agonistic or threatening interactions are as promoters of HPA function (Gust *et al.*, 1993a,b; Saltzman, Schultz-Darken, Scheffler, Wegner, and Abbott, 1994; Smith and French, 1997a).

When marmosets were housed in a novel cage alone, they exhibited behavioral changes indicative of distress and agitation. Levels of locomotory behavior

across the 4-day novel-cage housing and reunion phases were significantly elevated in animals housed in the novel cage alone, compared to under control conditions. In contrast, when marmosets were housed in the novel cage in the presence of the pairmate, rates of locomotion over the 4-day period did not vary from baseline levels. When animals were housed alone in the novel cage, their levels of locomotion on the first day in the unfamiliar cage were positively correlated with levels of urinary cortisol in the first void sample collected the following day. This result suggests that levels of locomotory behavior may provide a further noninvasive index of stress in marmosets in addition to levels of excreted cortisol. Increased locomotory activity in response to a stressor appears to be a generalized response in primates and is produced by a range of stressors including separation (Levine *et al.*, 1993), novelty (Hennessy *et al.*, 1995), and fear stimuli (e.g., a snake, Coe, Franklin, and Smith, 1982).

Another behavior indicative of stress, twitter vocalizations, was also modified by exposure to novelty. Twitter vocalizations were elevated in subjects on the first day of housing in a novel cage alone but there was no correlation between twitter frequencies and concentrations of urinary cortisol on this or any other day of the experimental manipulations. This result is in contrast to the positive correlation observed between locomotion and cortisol values during the first day of novel-cage housing. Previous studies assessing separation-induced distress have identified both a dissociation (Coe *et al.*, 1978, 1983; Levine *et al.*, 1993) and an association (Gunnar, Gonzalez, and Levine, 1980; Hennessy, 1987) between HPA activity and behavior. In our study, the close physical presence of the pairmate during the stressor eliminated the high rates of twitter vocalizations produced when animals were exposed to the stressor alone.

The manner in which scent marking behavior changed across experimental manipulations is consistent with their distress. Novelty has been shown previously to promote increased levels of scent marking behavior in callitrichids (see review in Epple, Belcher, Kuderling, Zeller, Scolnock, Greenfield, and Smith, 1993). The behavior exhibited by animals in the partner-present condition is consistent with this observation. Marking frequency was significantly elevated in our marmosets on Day 1 of the novel-cage housing and reunion phase. Animals exposed to novelty in the partner-absent condition failed to show increased scent marking in response to exposure to novelty. This latter results suggested that the presence of the heterosexual partner may facilitate the expression of species-typical behaviors when confronted with a novel environment.

In conclusion, the study has demonstrated HPA activation in marmosets in response to environmental novelty. Furthermore we have shown that the presence of the heterosexual pairmate reduces the physiological and behavioral consequences of novel-cage housing. In marmosets, interactions with heterosexual pairmates may function as homeostatic regulators of HPA function. If prolonged activation of the HPA axis above baseline levels has as deleterious health consequences in marmosets as has been demonstrated in other primate species (see reviews in Rivier and Rivest, 1991; Sapolsky, 1993; Coe, 1993), social attachments in marmosets may be important for an animal's well-being.

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