

RESEARCH ARTICLE

The Influence of Sex and Relatedness on Stress Response in Common Marmosets (*Callithrix jacchus*)NICOLE L. GALVÃO-COELHO^{1*}, HÉLDERES PEREGRINO A. SILVA¹, AND MARIA BERNARDETE CORDEIRO DE SOUSA²¹Department of Physiology, Universidade Federal do Rio Grande do Norte, Natal Rio Grande do Norte, Brazil²Department of Physiology and Graduate Program in Psychobiology, Universidade Federal do Rio Grande do Norte, Natal Rio Grande do Norte, Brazil

Research in stress physiology has demonstrated the benefits of receiving social support during stressful conditions. However, recent data have shown that the efficacy of social support in buffering physiological and behavioral responses to stressor agents depends on species, sex, and relatedness among animals. This study investigated whether different kinds of social support (presence of same sex related or nonrelated conspecifics) have the same effect on hormonal (fecal cortisol levels) and behavioral responses (agonistic: scent-marking and individual piloerection; anxiety: locomotion; tension-reducing: autogrooming, allogrooming, and body contact). We used adult male and female isosexual dyads of *Callithrix jacchus*, a small Neotropical primate from the Callitrichidae family, widely used in the study of stress and related diseases. Following a 28-day baseline phase, dyads faced three challenging situations (phase 1: dyads were moved together from the baseline cage to a similar new cage; phase 2: each dyad member was moved alone to a new cage; and phase 3: dyad members were reunited in the same baseline cage). Type of social support was found to influence the response to stressors differently for each sex. Related male dyads did not change their hormonal or behavioral profile over the three experimental phases, when compared to the baseline phase. For nonrelated male dyads, social support buffered hormonal but not behavioral response. For females, the social support offered by a related and nonrelated animal, does not seem to buffer the stress response, as shown by correlations between agonistic behaviors versus cortisol and locomotion during all three experimental phases and a significant increase in fecal cortisol levels during phases 2 and 3, when compared with baseline levels. The results only partially support the buffering model theory and corroborate other studies reporting that the benefits of social support during a period of crisis arise only when it is adaptive for that species. *Am. J. Primatol.* 74:819–827, 2012. © 2012 Wiley Periodicals, Inc.

Key words: stress; cortisol; social support; same sex dyads; common marmosets

INTRODUCTION

The social environment can be a source of stressor agents [Blanchard et al., 2001; Creel, 2001; Olsson & Westlund, 2007; Schapiro et al., 1998] or act as a salutary agent, buffering the stress response [Ditzen et al., 2008; Sachser et al., 1998]. In some hierarchically organized societies, such as those of olive baboon monkeys [*Papio anubis*, Sapolsky & Share, 2004], long-tailed macaque [*Macaca fascicularis*, Shively et al., 1997], and squirrel monkeys [*Saimiri sciureus*, Coe et al., 1979], subordinates are more exposed to parasites and subject to restricted food access and reproductive resources. By contrast, in callitrichid primates such as common marmosets (*Callithrix jacchus*) and cotton top tamarins (*Saguinus oedipus*), the costs of maintaining dominance are different for each sex and may affect the characteristics of physiological and behavioral response

to stress [Abbott et al., 2003]. Furthermore, among callitrichids, changes in social environment due to population density variations after emigration or immigration flows [Sousa et al., 2009], encounters between individuals or groups [Lazaro-Perea et al., 2000], artificial introduction of a new group member [Johnson et al., 1996], separation of the mother from

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her litter [Dettling et al., 2002], separation of reproductive pair members [Smith et al., 1998], or isosexual dyad members in isolation or together [Sousa et al., 2002], may trigger the autonomic and endocrine components of the stress response and modify behavioral profiles.

Despite undeniable evidence pointing to the costs of group living, many studies address its benefits and underscore the importance of social support during periods of crisis, since it reduces the harmful effects of chronic stress. This theory is known as the buffering model [Cohen & Wills, 1985]. Moreover, some studies also suggest that social support can prevent the development of physical and psychological pathologies on a daily basis, rather than only during a crisis, since it seems to promote welfare and enhance health. This theory is referred to as the main effect [Cohen & Wills, 1985; Stockdale et al., 2007].

Studies using human and animal subjects have shown that social support acts at a cognitive level to reduce the intensity of perceived stress, and at a physiological level to modulate the neuroendocrine stress response by reducing autonomic and hypothalamic-pituitary-adrenal (HPA) axis activity [House et al., 1988; Lepore, 1998; Seeman & McEwen, 1996; Smith et al., 1998]. For example, Gust et al. [1996] investigated the cortisol response of the rhesus monkey (*Macaca mulatta*) in a period of social instability during new group formation, observing a significantly smaller increase in cortisol levels in animals that had a social partner.

Several human studies have addressed the influence of social support on the stress response of adult subjects. Conversely, data are scarce for nonhuman primates, particularly those using experimental designs considering sexual differences. Most nonhuman primate investigations involve the mother–infant relationship [DeVries, 2003; Seeman & McEwen, 1996] or seek to demonstrate the relevance of social support in stressful situations, regardless of its quality [Gilbert & Baker, 2011; Gust et al., 1996].

Callithrix jacchus, a small Neotropical primate, is ideally suited for biomedical research [Mansfield, 2003] due to both behavioral and physiological aspects of its stress response [Galvão-Coelho et al., 2008; Norcross & Newman, 1999; Sousa et al., 2002]. In the natural environment, these animals live in cooperative breeding groups of 3 to 15 individuals. Although preferentially monogamous, some polygamous relationships have been observed [Yamamoto et al., 2010]. Subordinates act as helpers [Digby, 1995] and may undergo reproductive suppression [Sousa et al., 2005].

Some studies have demonstrated similarities between human and *C. jacchus* psychosocial stress response to socially stressful situations, such as mother–infant separation [Pryce et al., 2004, 2005],

heterosexual pair separation [Johnson et al., 1996; Leão, 2001; Norcross & Newman, 1999], stress reactivity to a new physical [Gerber et al., 2002] or social [Gerber & Schnell, 2004] environment, and prenatal stressors [Tauber et al., 2006]. In recent research, three different stress response profiles were identified in captive *C. jacchus*, widening the usefulness of this animal as a model in psychosocial studies of stress response [Galvão-Coelho et al., 2008]. However, the influence of social support and its modulators (sex and relatedness) on the behavioral and hormonal response to physical and psychological stressors has yet to be determined in this species. Therefore, the present study investigated whether *C. jacchus* males and females, maintained in isosexual dyads, differ in their reaction to stress as a function of the relatedness of the buffering partner facing intermediate levels of environmental and social stress. These include being moved to a new physical environment with the social partner, relocation to a new physical environment alone, and being reunited with the social partner in the initial physical environment after a period of separation.

METHODS

Maintenance

Fourteen isosexual adult *C. jacchus* dyads were used (males, $N = 6$ dyads; females, $N = 8$), half of which were relatives and half nonrelatives. Three of the four related female dyads were composed of mothers and daughters, and one of sisters. All related male dyads were twin brothers and information regarding sex, age, and relatedness of the animals is shown in Table I. Nonrelative male and female dyads were paired 10 to 20 days before the beginning of the study. Related dyads were retrieved from their family groups 30 to 60 days prior to starting the investigation. Each dyad was then housed in a new cage. As demonstrated in earlier studies [Abbott, 1984; Abbott et al., 1988], behavioral stabilization of dominance hierarchy for same-sex common marmosets in newly formed peer groups occurs in the first three days after pairing, for both males and females. As such, we can assume that the minimum 10-day period imposed prior to commencing the present investigation, where animals were under controlled conditions, may be sufficient to stabilize the relationship between members of nonrelated dyads.

All animals were living in the Primatology Center at the Federal University of Rio Grande do Norte, housed in outdoor brick cages (2.0 m × 2.0 m × 1.0 m) under natural light, humidity, and temperature conditions. Cages were equipped with a nest box, boards, branches, and a one-way mirror on the front wall, through which behavioral observations were made. Nonrelated dyads had no social or visual contact

TABLE I. Animal Information

Dyads	Relatedness	Age (months) at beginning of the experiment
Females		
Related		
822/960	M/D	64/55
836/972	M/D	65/15
800/974	M/D	72/15
912/928	S	38/31
Nonrelated		
902/940	–	36/22
844/896	–	56/42
916/920	–	32/30
906/712	–	78/58
Males		
Related		
779/781	T	21
869/871	T	24
775/777	T	24
Nonrelated		
874/883	–	29/23
765/791	–	67/64
995/1045	–	32/26

M/D: mother and daughter; S: sisters; T: twins.

before the beginning of study, although auditory or olfactory contact was possible. Water was available ad libitum and meals were offered three times a day. All experimental animals were habituated to the presence of researchers collecting feces on the floor of the cage before the beginning of observation sessions.

Protocols utilized in this study were approved by the local university animal care committee (UFRN–Universidade Federal do Rio Grande do Norte) according to Brazilian Guidelines for the care and use of animals in scientific research and also adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

Experimental Design

The study consisted of four successive phases, baseline plus three different experimental manipulations in social and/or physical environments, as stressful stimuli. The baseline phase lasted four consecutive weeks (28 days) and the remaining phases 1 week each. For all phases, behavior was recorded for 30 min between 06:30 a.m. and 09:30 a.m. to avoid the influence of circadian rhythms on both behavioral and hormonal variables [Sousa & Ziegler, 1998]. During the baseline phase, behavioral and hormonal (feces samples) data were collected on alternate days and in the other three phases on a daily basis.

TABLE II. Definition of Animal Behaviors Quantified in Terms of Duration or Frequency

Behaviors	Description	Type of measure
Social		
Allogrooming	Act of grooming the partner's fur	Duration
Contact	To be in bodily contact with the partner.	Duration
Simultaneous piloerection	To arch and bristle the body and tail fur, walking together with the partner.	Frequency
Individual		
Locomotion (approach/withdrawal)	Number of approaches or withdrawals between each animal of the dyad.	Frequency
Autogrooming	Act of grooming their own fur.	Duration
Individual piloerection	To arch and bristle the body and tail fur.	Frequency
Scent-marking	Act of rubbing the anogenital or supra-pubic regions against the substrate.	Frequency

Baseline phase (B): A period of 28 days before phase 1, where data were collected to establish a behavioral and hormonal profile.

Phase 1 (P1–Paired in a new environment): Dyads were moved together to a new cage, similar to the one they inhabited during the baseline phase.

Phase 2 (P2–Alone in a new environment): Dyads were separated and each animal was moved alone to a new cage, similar to those used in previous phases, without olfactory or visual contact with the social partner.

Phase 3 (P3–Reunion): Previously separated dyad members were reunited in the original baseline phase cage.

Behavioral Data

Behavioral data collections were performed through the one-way mirror by four observers. Interobserver accuracy related to behavioral records was 92%. The continuous focal sampling method [Altmann, 1974] was used, and the frequency and/or duration of behaviors exhibited by each focal animal during a 3-min period were recorded. A description of each behavior, adapted from an ethogram [Stevenson & Poole, 1976], is presented in Table II.

The behaviors of autogrooming, scent-marking, and individual piloerection were registered in all study phases. Allogrooming, simultaneous piloerection, body contact, approaches, and withdrawals

were also recorded during the phases in which animals were paired (B, P1, and P3).

Hormonal Data

Fecal collection for cortisol measurement was performed between 06:30 a.m. and 09:30 a.m. to avoid influencing the circadian profile [Raminelli et al., 2003]. Before starting data collection, the cage floor was cleaned and all animals were observed until defecation occurred. Only samples for which the collector could ensure the donor's identity were collected, using a metal spatula. Samples were stored in small, labeled glass tubes with snap caps and frozen immediately at -20°C until measurement. Cortisol extraction was performed using hydrolysis and solvolysis procedures, following Sousa and Ziegler [1998] protocols. In common marmosets, the time lag for release of cortisol into plasma and subsequent excretion in feces was assumed to be approximately 8–12 h, as described by Sousa and Ziegler [1998]. Fecal concentrations of cortisol were measured using enzyme immunoassay (ELISA) in ng/g of wet fecal samples. Intra- and interassay variation coefficients were 3.8% and 21.9%, respectively.

Statistical Analysis

For all experimental phases, cortisol concentration values obtained in fecal samples were log-normalized and data analyzed using the general linear model (GLM) and post hoc Fisher's least significant difference (LSD) test. Previous exploratory data analyses showed that males and females exhibited different frequency distribution in hormonal values, resulting in a bimodal curve when data for both sexes were considered jointly. As a result, and taking into account different sample sizes, male and female data were analyzed separately, in accordance with GLM test requirements. The dependent factor "phases" was considered as the four levels of repeated measures (Baseline, Phase 1, Phase 2, and Phase 3). "Sex" (two levels; males and females) and "relatedness" (two levels: relative and nonrelative) were the independent factors in the model.

Since both endocrine and behavioral data were collected on alternate days during the baseline phase and daily during the other phases, 14 samples were obtained for each individual in the baseline phase and 7 in the remaining experimental phases. Therefore, for each individual, hormonal values obtained from two consecutive collections in the baseline phase were grouped and a mean was calculated, resulting in seven sample values per subject in this phase. This procedure was necessary in order to apply statistical tests of repeated measures, which requires the same sample size for different stages of the dependent variable (phases).

As previously described for hormonal data, behavioral values obtained from each two consecutive collections in the baseline phase were grouped into seven sample values for statistical analysis. Data of each individual were summed within each of the four phases to be analyzed. Given that behavioral data were not normally distributed, nonparametric Friedman ANOVA and Wilcoxon tests were applied in order to analyze the "phase" effect. The Mann-Whitney test was used to assess the effect of "sex" and "relatedness."

Spearman's correlation test was applied to determine whether or not there were correlations between cortisol concentrations and frequencies or behavior duration, when the three experimental phases data were considered jointly. Behaviors were grouped into three categories, (i) agonists: the sum of the frequencies of individual scent-marking and piloerection, (ii) anxiety: locomotor activity: sum of frequencies of approaches and withdrawals, and (iii) tension-reducing behaviors: the sum of the duration of autogrooming, allogrooming, and body contact.

Considering the 8–12 h time lag for excretion of cortisol in feces, the animals were moved to a new environment in the afternoon and fecal sampling was performed the morning after this change. It is therefore expected that cortisol concentration in morning fecal samples will reflect hormonal modifications caused by experimental manipulation of the previous day. Thus, statistical correlation tests between behavioral and cortisol data were conducted using the values of behaviors matched with cortisol values from the following day.

For all tests, a two-tailed P -value was defined as statistically significant when $P \leq 0.05$.

RESULTS

Male Dyads

Endocrine response

In the baseline phase, similar fecal cortisol levels were found between related and nonrelated male dyads (LSD test, $df = 76$; $P = 0.48$). However, hormone levels differed among the three experimental phases (GLM test: $df = 76$; $F = 3.14$; $P < 0.05$), with significantly higher cortisol levels only for nonrelated males when compared to related individuals during phase 2 (LSD test, $df = 76$; $Rm \times NRm$: [B; $P = 0.48$]; [P1: $P = 0.63$]; [P2: $P < 0.01$]; and [P3: $P = 0.09$]) (Fig. 1). Data analysis of related male dyads showed no significant changes in fecal cortisol levels across successive experimental phases when compared with baseline (LSD test, $df = 76$: [B \times P1; $P = 0.96$]; [B \times P2; $P = 0.96$]; [B \times P3; $P = 0.14$]) or among experimental phases (LSD test, $df = 76$: [P1 \times P2; $P = 0.99$]; [P1 \times P3; $P = 0.13$]; [P2 \times P3; $P = 0.13$]) (Fig. 1). By contrast, for nonrelated dyads, a significant increase in fecal cortisol levels occurred when

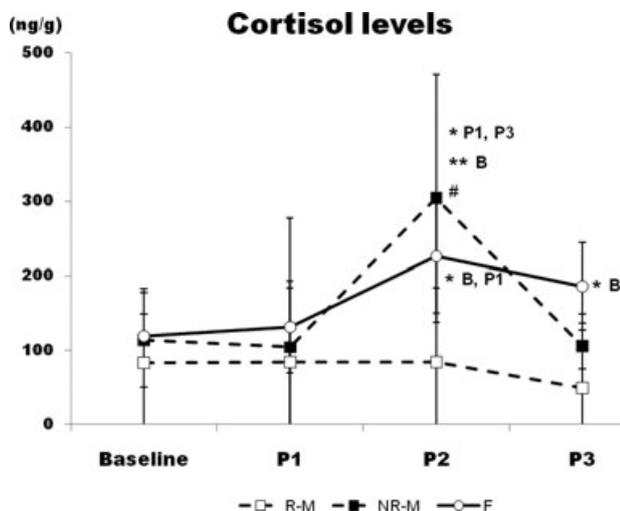


Fig. 1. Mean (\pm SEM) cortisol for male dyads, relatives (R-M) and nonrelatives (NR-M), and females dyads (F) [relatives and nonrelatives pooled] of *Callithrix jacchus*, in each phase of the study (B, baseline phase; P1, new environment paired; P2, new environment isolated; and P3, reunion). GLM statistical test for repeated measures, $P < 0.05$. *Significant difference in cortisol levels between the respective study phase and those represented by the number beside the symbol. **Tendency toward a significant difference in cortisol levels ($0.05 < P < 0.09$) between the respective study phase and those represented by the number beside the symbol. #Significant difference in cortisol levels between related and nonrelated males dyads in the respective study phase.

animals were moved to a new cage alone (phase 2). During this phase, fecal cortisol levels were significantly greater than those of phase 1 (LSD test, $df = 76$; $P2 \times P1: P < 0.05$) and phase 3 (LSD test, $df = 76$; $P2 \times P3: P < 0.05$). Moreover, for nonrelated male dyads, a statistical tendency of increased cortisol was recorded when phase 2 and baseline cortisol levels (LSD test, $df = 76$; $B \times P2: P = 0.065$) were compared. Data analysis of nonrelated male dyads showed no significant changes in fecal cortisol levels when P1 and P3 were compared with baseline (LSD test, $df = 76$; [$B \times P1: P = 0.86$]; [$B \times P3: P = 0.88$]).

Behavioral response

When analyzing type of social support, related male dyads showed a significant increase in time spent in body contact during phase 1 when compared to the baseline phase (Wilcoxon test: $df = 11, z = 2.20$; [$B \times P1: P < 0.05$, baseline median = 8.5, $Q^{25} = 0, Q^{75} = 58/P1$ median = 130, $Q^{25} = 36, Q^{75} = 526$]). For these dyads, when the three experimental phase data were pooled, significant negative correlations were found between tension-reducing behaviors and cortisol (Spearman correlation: $r_s = -0.47; P < 0.05$) and locomotion ($r_s = -0.2; P < 0.05$) as shown in Table III.

Nonrelated male dyads (NRm) exhibited a significantly higher frequency of locomotor activity than

TABLE III. Correlations between Behavior \times Behavior and Behavior \times Cortisol, Considering Pooled Data of the Three Experimental Phases in Common Marmoset Dyads

Variables	Dyads			
	Related females	Nonrelated females	Related males	Nonrelated males
C \times A	0.27 ^a	0.16 ^a	0.06	0.07
C \times TR	0.03	0.00	-0.47 ^a	0.03
C \times L	0.27	-0.01	-0.05	-0.1
A \times TR	-0.02	-0.05	-0.05	-0.02
A \times L	0.53 ^a	0.54 ^a	-0.04	-0.56 ^a
TR \times L	-0.01	-0.24	-0.2 ^a	-0.37 ^a

A: agonistic behavior; L: locomotion; TR: Tension-reducing behaviors and C: cortisol, ^aSpearman correlation test, $P < 0.05$.

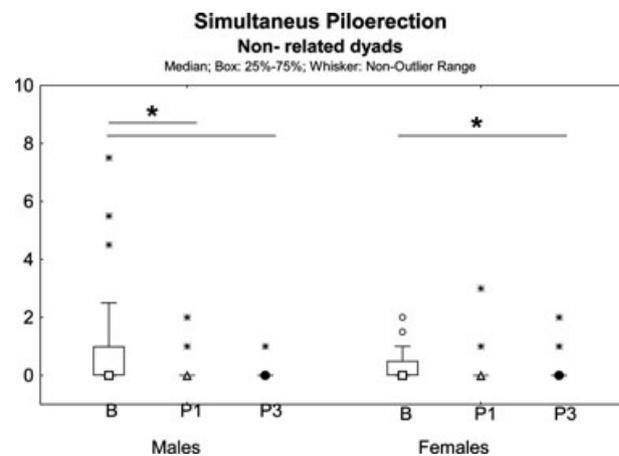


Fig. 2. Box plots of simultaneous piloerection for nonrelated male and female dyads of *Callithrix jacchus* at each phase of the study (B, baseline phase; P1, new environment paired; and P3, reunion). Friedman ANOVA, $P < 0.05$. *Significant difference in cortisol levels between the respective study phases.

related male (Rm) dyads during the baseline phase (approach: Mann-Whitney test: $df = 11, U = 3.00; P < 0.01$, [Rm: median = 2, $Q^{25} = 1, Q^{75} = 4$], [NRm: median = 7.7, $Q^{25} = 5, Q^{75} = 12.5$]) (withdrawal: Mann-Whitney test: $df = 11, U = 1.00; P < 0.05$, [Rm: median = 1, $Q^{25} = 1, Q^{75} = 4$], [NRm: median = 8.7, $Q^{25} = 6.5, Q^{75} = 11$]). Nonrelated dyads showed a significant reduction in simultaneous piloerection during phases 1 and 3, when compared with the baseline phase (Friedman ANOVA: $df = 11, \chi^2 = 6.53; P < 0.05$, [$P1 \times B; P < 0.05$]; [$P3 \times B; P < 0.05$]) (Fig. 2). They also exhibited a significant negative correlation between tension-reducing and agonistic ($r_s = -0.56; P < 0.05$) and locomotion behaviors ($r_s = -0.37; P < 0.05$), when data from the three experimental phases were considered jointly (Table III).

Female Dyads

Endocrine response

For females, type of social support (related or nonrelated) showed no significant differences in fecal cortisol levels across the three experimental phases (GLM test: $df = 101$, $F = 2.18$; $P = 0.09$). We pooled these dyads in order to analyze statistical hormonal response across the study phases. Compared with baseline levels, both related and nonrelated dyads displayed significantly raised cortisol levels in both phases 2 and 3 (LSD test, $df = 101$: [$P1 \times B$; $P = 0.64$]; [$P2 \times B$; $P < 0.01$]; [$P3 \times B$; $P < 0.05$]). Additionally, fecal cortisol levels measured in phase 2 were significantly higher than those of phase 1, for both types of dyads (LSD test, $df = 101$: [$P2 \times P1$, $P < 0.05$]; [$P2 \times P3$, $P = 0.1$]), as illustrated in Figure 2.

Behavioral response

Nonrelated female dyads (NRf) showed a significant reduction in simultaneous piloerection during phase 3, when compared with the baseline phase (Friedman ANOVA: $df = 15$, $\chi^2 = 2.22$; $P < 0.05$; [$P3 \times B$; $P < 0.05$]) (Fig. 2).

Correlation between behaviors and behaviors \times cortisol were made for nonrelated and related females dyads (Rf) separately. A significant positive correlation between agonistic behaviors and locomotor activity was found (Table III) (Spearman correlation: Rf, $r_s = 0.53$, $P < 0.05$; NRf, $r_s = 0.54$; $P < 0.05$). Furthermore, a weak significant positive correlation was recorded between cortisol and agonistic behaviors for both dyads (Spearman correlation: Rf, $r_s = 0.27$, $P < 0.05$; NRf, $r_s = 0.16$; $P < 0.05$), as shown in Table III.

DISCUSSION

Results of the present study demonstrate that relatives and nonrelatives differ in their buffering power and buffering ability differs between common marmoset males and females. Evidence is presented to show that cortisol levels did not change in related males across the three experimental phases, although an increase in time spent in contact was observed immediately after animals were moved to a new cage (phase 1). Additionally, these dyads displayed antianxiety behaviors in phases 1 and 3, evidenced by the negative correlation between tension-reducing behaviors and cortisol levels and locomotion. Autogrooming, allogrooming, and contact were considered tension-reducing behaviors, given that they stimulate oxytocin secretion, which in turn reduces HPA axis function [DeVries et al., 2003, 2007; Detillion et al., 2004; Wittig et al., 2008]. Efficient use of these behaviors may be responsible for the more stable cortisol level profile exhibited by related male dyads. Conversely, nonrelated males showed

a statistically significant increase in cortisol levels when moved alone to a new cage (phase 2), compared to phases 1 and 3. Moreover, these dyads displayed a statistical tendency to significantly increased cortisol levels when phase 2 and baseline phase were compared. During phase 2, cortisol levels of nonrelated males were also significantly higher than those exhibited by related males.

Thus, social support, whether provided by a related or nonrelated conspecific, had a different influence on behavioral and/or hormonal stress response in common marmoset males. These results suggest that, for males, the presence of a same-sex related or nonrelated conspecific can buffer hormonal modifications to new environments, since no increases in cortisol levels were observed in either male dyad in phases 1 and 3.

Norcross and Newman [1999] reported that, for *C. jacchus*, the presence of social support provided by a nonrelated conspecific attenuates cortisol stress response, when compared with the response of isolated animals. Smith et al. [1998] also observed that *Callithrix kuhli* housed in a new cage alone showed elevated urinary cortisol levels, but no increase was recorded when animals were exposed to a new environment with their pair mates.

In the present study, the increase in cortisol levels exhibited by nonrelated males when isolated may result from higher levels of social competition between nonrelated animals than related ones, as well as the expectation of subsequent new group formation. The main effect theory [Cohen & Wills, 1985] proposes that the benefit of adequate social support operates continually among individuals, even in the absence of the partner, given that it promotes stability in physiological, psychological, and behavioral parameters. According to our data, this long-lasting stability may have contributed to the steady cortisol levels and behavioral profile observed for related male dyads during social isolation. Moreover, these findings strengthen the discussion in terms of genetic variation, since as demonstrated by Ross et al. [2007], some common marmoset sperm cells carry chimeric genes of the twin acquired in the maternal uterus. These genes can be passed on to the offspring, given that gonadal chimerism does not lead to sterility in this species. Thus, this genetic particularity may be involved in the evolution of male reproductive strategies, which could include high levels of cooperation between twin brothers. Studies addressing emigration patterns in wild *C. jacchus* report that isolated females emigrated more frequently than males from the natal group [Sousa et al., 2009], and that two related males and an emigrant female constitute the basis for new group formation [Araujo, 1996]. Recent genetic research using mitochondrial DNA markers (to examine the distribution of matriline) in free ranging *C. jacchus* groups has shown that the relatedness coefficient among males and the

reproductive female is low [Faulkes et al., 2009], whereas high relatedness among males was found using microsatellite markers [Nievergelt et al., 1998]. These data strongly suggest that in wild *C. jacchus* groups, males are commonly immigrants and related to one another. Thus, twin males might not perceive social privation as severely as nonrelated males, since they do not compete with one another to achieve reproductive success. The adaptive implications of the twin male bond in *C. jacchus* may justify the long-lasting buffer effect of male twin presence on stress response, observed in phase 2 of the present study, reinforcing the main effect theory. However, given that our experimental phases lasted only 7 days, it is not possible to determine the duration of this buffer effect.

For nonrelated male dyads, social support between same-sex conspecifics was not efficient in buffering the behavioral response to experimental stressors. This was evidenced by a significant decrease in simultaneous piloerection compared to baseline levels when animals were moved together to a new cage, and when they were reunited after isolation. This declining response might indicate a reduction in affinity and synchronicity between members of the dyad when facing a territorial defense context, as suggested by Silva and Sousa [1997].

For *C. jacchus* females, both types of female dyads [related and nonrelated] experienced a significant increase in cortisol levels when moved alone to a new cage and after reunion with their partners, in addition to exhibiting behavioral changes indicative of distress across all experimental phases. Nonrelated female dyads showed a significant reduction in simultaneous piloerection during phase 3, when compared with the baseline phase. Both female dyads [nonrelated and related] displayed significant positive correlations between agonistic behaviors (individual scent-marking and piloerection), cortisol, and locomotor activity (anxiety) during all experimental phases. It is therefore possible to speculate that natural competition between females overshadows the type of social support, whether supplied by a relative or nonrelated same-sex conspecific. Our findings corroborate those of Johnson et al. [1996], who reported an increase in cortisol levels among common marmoset females when they were separated from their social partner and remained isolated. This increase continued even when new social groups were formed. In other words, the presence of a same-sex conspecific does not buffer the stress response, contrary to what we found for males and what has been reported for breeding pairs of Wied's marmosets [Smith et al., 1998]. Common marmoset females also react differently to the degree of familiarity with males. They experience decreased cardiac frequency and locomotor activity when moved to a new environment with their sexual partners, but not when accompanied by a nonfamiliar male [Gerber et al., 2002].

Sex differences in stress reactivity seem to be part of an adaptive strategy related to dispersion mechanisms in this species. It is well documented that in the wild, *C. jacchus* females tend to disperse and establish hierarchical relationships in new groups more than males do [Araujo, 1996; Sousa et al., 2009]. Females may therefore be capable of facing challenges in the physical and social environment, exhibiting a more competitive behavioral profile than males so as to achieve reproductive success, even under unfavorable conditions. Moreover, subordinate females may escape from reproductive suppression imposed by the dominant female, as reported in Saltzman et al. [1997], Ziegler and Sousa [2002], and Yamamoto et al. [2010], causing even greater tension in intrasexual relationships inside groups. By contrast, male social bonding represents the core of groups [Araujo, 1996; Yamamoto et al., 2010].

Our results reinforce the need to consider the animal's sex when analyzing the effect of social buffering on stress response, avoiding generalization of results to one species if the experimental protocol takes only one sex into account. They also identify the need to measure behavioral and physiological variables concomitantly, since dissociation between behavioral and hormonal parameters may occur. This type of dissociation is frequently observed in stress studies, demonstrating that behavior is not always a clear indication of a subject's physiological condition [Hennessy et al., 2008; Norcross & Newman, 1999].

As such, the present results only partially support the buffering model theory, which states that the presence of social support per se attenuates the harmful effects of stress exposure [Cohen & Willis, 1985]. By contrast, we observed that both types of social support (whether supplied by a relative or nonrelated same-sex conspecific) and sex influenced the hormonal and behavioral response to physical (new cage) or social (separation and reunion) stressors. Several human and rodent studies corroborate our results, showing that not all types of social support are equally efficient in buffering the stress response to a challenge. Thus, some factors, such as relatedness, familiarity, and affiliation between animals, as well as sex-specific behavioral and endocrine patterns, may influence the effectiveness of social support [Kirschbaum et al., 1995; Lepore, 1998; Vaananen et al., 2005]. This indicates the importance of considering these variables in order to reduce undesirable negative stress effects, mainly for species such as common marmosets, which are potential biomedical models for stress studies.

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