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ORIGINAL ARTICLE

Behavioral and hormonal response of common marmosets, *Callithrix jacchus*, to two environmental conditions

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Abstract Captive animals of several species change their behavioral pattern and hormonal profile in response to physical (for example, cage size and temperature) and social (for example, group size and social isolation) modification of their environment. To evaluate the effect of environmental change in captivity, the affiliative (contact/proximity and allogrooming) and individual (approach, leaving, scent mark, locomotion, and autogrooming) behavior of five family groups of common marmosets, *Callithrix jacchus*, were recorded on weekdays (non-quiet) and at the weekend (quiet) for two months. In addition, fecal samples were collected for four of these groups to measure their cortisol levels under both conditions. The behavioral pattern and hormonal profile of breeding pairs and their offspring were modified by different management routines used in the experimental conditions. We found that the animals spent more time in affiliative interactions at the weekend, whereas on weekdays, they showed more individual behaviors. Moreover, cortisol levels of breeding pairs and their offspring were higher on weekdays, suggesting that common marmosets living in captivity react to environmental modification by changing their behavioral and hormonal pattern.

Keywords *Callithrix jacchus* · Common marmoset · Captivity · Management routine · Cortisol

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Introduction

Numerous studies have found effects of environmental conditions on the behavioral and physiological responses in several groups of animals living in captivity. Physical differences such as cage size, temperature, illumination, and social changes, including group size, social isolation, and the presence of strangers, affect the behavioral pattern of chickens (Bizeray et al. 2002), canids (Neamand et al. 1975), and nonhuman primates (Box 1988; Johnson et al. 1991; Box and Rohrhuber 1993; Gaspari et al. 2000). In common marmosets (*Callithrix jacchus*), Scott (1991) found a difference in locomotion between members of a family group that was related to changes in the illumination level of their cages. Mota (1999) found that environmental changes (cage size, temperature, humidity, and food-offer schedule) affected the sociosexual interaction between male and female common marmosets in two captive colonies.

The presence of intruders in an animal's physical environment can also lead to alterations in the behavioral pattern of primates in the form of agonistic response. Studies using callitrichids have shown that breeding pairs of cotton-top tamarins (*Saguinus oedipus*) and common marmosets (*Callithrix jacchus*) exhibit an increase in scent marking and agonistic behaviors in the presence of non-related conspecifics (Epple 1970; French and Snowdon 1981). Similarly, Chamove et al. (1988) and Hosey (2005) studied the effect of zoo visitors on the behavioral pattern of primates, and found that animals were less affiliative, more active, and more aggressive when visitors were present. The presence of intruders of the same and different species in their social environment leads to a change in the behavioral response of male and female primates that seems to be associated with the maintenance of social stability. On the other hand, under non-zoo conditions, three macaque species behaved differently after being exposed to a passive observer (no interaction), showing a fearful, aggressive, or affiliative response (Clarke and Mason 1988); however, they appear to habituate after regular contact with humans.

Taking into account that animals respond behaviorally to physical and social environment variation, it is expected that housing conditions and management routine in captivity also lead to changes in physiological factors that enable the animal to deal with it, for example the release of peptides and steroids associated with the response to stress (Manteuffel and Puppe 2000). The main glucocorticoid found in primates is cortisol, which is released during behavioral stress (Sapolsky 1993). Cortisol assays have been widely used to determine the effects of both acute (Smith and French 1997) and chronic stress on captive primates, including alterations in captive conditions (Johnson et al. 1991; Crockett et al. 2000; Cross et al. 2004) and maternal separation or peer group formation (Terao et al. 1995). Moreover, studies with non-human primates showed a relationship between elevated cortisol and social deprivation (*Callicebos moloch*: Mendoza and Mason 1989), reduction of physical space (*Macaca mulatta*: Hassler et al. 1989) and exposure to novel environments (*Saimiri sciureus*: Mendonza 1991).

In the common marmoset, Peters (2001) found a relationship between high cortisol levels and variations in temperature, lighting, physical space available, and the presence of noise near the primate colony that might have an effect on the animal's welfare. Additionally, Sousa and Ziegler (2000) showed an elevation in cortisol fecal levels in adult females after separation from their social group and pairing with a strange male. Newly-formed heterosexual pairs of the common marmoset showed a variation in fecal cortisol levels related to social environment changes (pair formation, separation and pair reunion) (Leão 2001). Moreover, Coelho (2005) found a significant negative correlation (−0.88) between time spent in contact with a family group member and cortisol levels in related male common marmosets, and a positive (0.83) correlation between cortisol and allogrooming in non-related individuals when transferred from their home-cage to a new one. This suggests that fecal samples can be used to evaluate the relationship between the hormonal response and changes in physical environment for this primate species.

Thus, the objective of this study was to evaluate the effect of environmental change on the behavioral pattern and fecal cortisol levels of captive social groups of common marmosets. We expected that:

1. the affiliative and individual behavior of the breeding pair and their offspring would vary between weekends and weekdays; and
2. there would be an increase in cortisol fecal levels related to the difference in routine management between both conditions in the primate colony.

Methods

Animals

The study involved five family groups of common marmosets (*Callithrix jacchus*). Each family group was formed by a breeding pair and their descendant offspring, as shown in Table 1. The animals were observed between January 2002 and February 2004. All were captive-born, except two adult males and one adult female, which were wild-caught as infants (Yamamoto 1993). They were housed in the breeding colony of the Physiology Department of Universidade Federal do Rio Grande do Norte, Natal-RN, Brazil (05°46′ S 35°12′ W).

Table 1 Information about the observed family groups

Group no.	Years of pair formation	Group size	Group composition (age year:month)		
			Adult breeding pair	Subadult	Juvenile
1	2	5	M571 (2:9)	M865(1:0)	M891 (0:6)
			F554 (4:10)		M893 (0:6)
2	1	4	M707 (4:5)		M931 (0:7)
			F638 (4:11)		M933 (0:7)
3	1	4	M899 (4:8)		F896 (0:9)
			F546 (4:11)		M971 (0:5)
4	2	5	M915 (4:7)	M917(1:0)	M961 (0:7)
			F686 (4:11)		M962 (0:7)
5	1	4	M819 (2:6)		M975 (0:5)
			F838 (3:9)		M977 (0:5)

M, male; *F*, female; *three digits*, individual code

Each family group was kept in a wire mesh and brick wall outdoor cage measuring 2.00 × 2.00 × 3.00 m under natural light–dark cycles. Cages were provided with a nest box, wooden platforms, branches, and roost. Families were visually and physically isolated from the other members of the colony. The animals were fed twice a day around 8:00 am with a protein mixture (powdered milk, corn syrup, eggs, bread, soy bean protein, and bone flour supplemented with vitamins A, D, and E) and around 2:00 pm with a portion of regional tropical fruits. Water was provided ad lib.

Experimental design

This study compared the behavioral pattern and hormonal profile of the animals under two conditions: weekday (non-quiet), when management routines and other handling activities were performed, and weekend (quiet), when feeding the animals at 8:00 am was the only routine activity performed in the breeding colony. The management routine on weekdays included animal feeding, and cage cleaning, and capture for purposes of weighing, medicating, or transporting. However, except for feeding, none of these activities was performed with the family groups on the observation session day (Wednesday) but they might be taking place in the surrounding cages. We chose Wednesday and Sunday as experimental conditions, based on the differences in the colony routine between these days. Affiliative behaviors (*contact and proximity*: time spent in body contact and within 30 cm of a family group member; *allogrooming*: time spent licking, picking at, or parting the hair of another animal with the fingers; and *approach and leaving*: frequency of going towards and away from an imaginary boundary of 15 cm from a family member, for at least 3 s) and individual behaviors (*scent mark*: frequency of rubbing the anogenital region on a surface of the cage; *locomotion*: the cage was divided into 60 quadrants of 38 cm each and the frequency of movement between two quadrants by the focal animal was recorded as one movement; and *autogrooming*: time spent licking, picking at, or parting his or her own fur with the fingers) were recorded. Each group was observed by two observers on both days of the week for 2 h for eight consecutive weeks, using the focal continuous method ($n = 32$ h/animal). Several studies performed in captivity and in the field show that the behavioral pattern of Callitrichidae is differently distributed throughout the day. According to these studies, marmosets and tamarins leave their sleeping sites after dawn and the first two hours of activity involve intensive foraging which is followed by resting, when social interaction, for example grooming, occurs (Sussman and Kinsey 1984; Stevenson and Rylands 1988). Moreover, a circadian rhythmicity was found for locomotor activity, with peaks in the morning and afternoon characterizing a bimodal pattern in captive common marmosets, and autogrooming behavior lasts longer in the morning (Menezes et al. 1993). In these senses, a two-hour interval for behavioral recording was adequate because it was long enough to describe the exploratory and social activities of family groups and their responsiveness to environmental change. No difference was found in the observers' recordings (Kendall's coefficient: $\tau = 0.92$, $P \leq 0.05$). The study was developed according to the animal welfare guidelines of Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil.

Fecal sampling

To determine cortisol levels we collected fecal samples over two months from four family groups. The use of feces over blood sampling has advantages, because the former is a non-invasive method that does not require handling and animal immobilization during sample collection (Dettmer et al. 1996). Samples were collected between 5:30 and 7:30 am on the day following the behavioral observation. Breeding pairs and offspring body parts (e.g., ear-tuffs, head, tail, or back) were marked using picric acid (yellow) in order to identify each individual's fecal sample in a family group. The sample was then placed in a plastic tube labeled with the animal, collection date, and time, and frozen at −20°C.

Cortisol assay

Fecal steroids were separated from the 0.1 g of fecal solids using the extraction procedures of hydrolysis and solvolysis described by Sousa and Ziegler (1998). Following the extraction process, fecal cortisol was quantified by an ELISA method (Munro and Stabenfeldt 1984). The coefficients of intra-assay and inter-assay variation were 15.49 and 3.88%, respectively.

Statistical analysis

Non-parametric and parametric tests were used. The behaviors (duration and frequency) were averaged across individuals. Differences in affiliative and individual behavioral categories of breeding males and females and their offspring between weekday and weekend conditions were compared by use of the Wilcoxon matched-pairs signed-ranks test (Z). We performed a log transformation before statistically analyzing cortisol levels. A dependent t-test was used to compare the cortisol levels of experimental animals between weekday (non-quiet) and weekend (quiet), and an independent t-test was performed to evaluate the effect of sex and experimental condition on the hormonal profile. Statistical significance was set at $P \leq 0.05$.

Results

Affiliative behavior

Social behaviors changed between weekdays (non-quiet) and weekends (quiet) as shown in Fig. 1. Time spent in contact/proximity decreased for all family group members on weekdays (*breeding pair*: male: $Z = 5.12$, $P \leq 0.05$, $N = 5$; female: $Z = 5.53$, $P \leq 0.05$, $N = 5$; *offspring*: subadult: $Z = 2.24$, $P \leq 0.05$, $N = 2$; juvenile: $Z = 3.10$, $P \leq 0.05$, $N = 10$). Allogrooming, however, decreased only in the adult animals (*breeding male*: $Z = 2.67$, $P \leq 0.05$; *breeding female*: $Z = 3.57$, $P \leq 0.05$). The approach frequency of subadult offspring ($Z = 2.15$, $P \geq 0.05$) was not affected by these differences (Fig. 1). For all group members, the highest frequency of leaving was found on weekdays (*breeding male*: $Z = 4.10$, $P \leq 0.05$; *breeding female*: $Z = 3.40$, $P \leq 0.05$; *subadult*: $Z = 2.11$, $P \leq 0.05$; *juvenile*: $Z = 3.25$, $P \leq 0.05$).

Individual behavior

Individual behaviors were also influenced by differences in management routine between observation days as illustrated in Fig. 2. For all group members, the highest frequency of scent mark and locomotion were found on weekdays, as shown in Fig. 2 (*scent marking*: breeding male: $Z = 6.20$, $P \leq 0.05$; breeding female: $Z = 6.35$, $P \leq 0.05$; subadult: $Z = 2.87$, $P \leq 0.05$; juvenile: $Z = 5.64$, $P \leq 0.05$; *locomotion*: breeding male: $Z = 8.14$, $P \leq 0.05$; breeding female: $Z = 8.32$, $P \leq 0.05$; subadult: $Z = 7.32$, $P \leq 0.05$; juvenile: $Z = 6.78$, $P \leq 0.05$). However, autogrooming increased for breeding pairs on weekdays (*breeding male*: $Z = 2.78$, $P \leq 0.05$; *breeding female*: $Z = 2.59$, $P \leq 0.05$) (Fig. 2).

Fecal cortisol

Fecal cortisol levels of breeding pairs (*male*: $t = -0.85$, $P \geq 0.05$: *female*: $t = -1.91$, $P \geq 0.05$) and offspring (*subadult*: $t = -1.80$, $P \geq 0.05$; *juvenile*: $t = -1.62$, $P \geq 0.05$) did not differ between non-quiet and quiet conditions. However, all group members' mean hormone levels were higher on weekdays (*breeding male*: 55.93 ± 9.19 ng/g; *breeding female*: 99.88 ± 17.77 ng/g; *subadult*: 74.17 ± 29.19 ng/g; *juvenile*: 60.47 ± 9.68 ng/g) than at weekends (*breeding male*: 47.22 ± 9.14 ng/g; *breeding female*: 76.13 ± 10.51 ng/g; *subadult*: 64.39 ± 21.2 ng/g; *juvenile*: 49.15 ± 9.89 ng/g). The interaction between animal category and experimental condition factors showed a significant difference between breeding male and female cortisol levels on weekdays with females having the highest cortisol levels ($t = 2.38$, $P \leq 0.05$). Concentrations of fecal cortisol in breeding females were higher than in other group members under both conditions.

Discussion

Environmental change has been accepted as a factor that modifies the distribution of the behavioral pattern of marmoset family groups. The frequency of approach, leaving, scent mark, locomotion, and time spent in autogrooming increased on weekdays whereas time spent on contact/proximity decreased. A similar effect of management routine was reported by Barbosa and Mota (2004) for breeding pairs of common marmosets. They found an

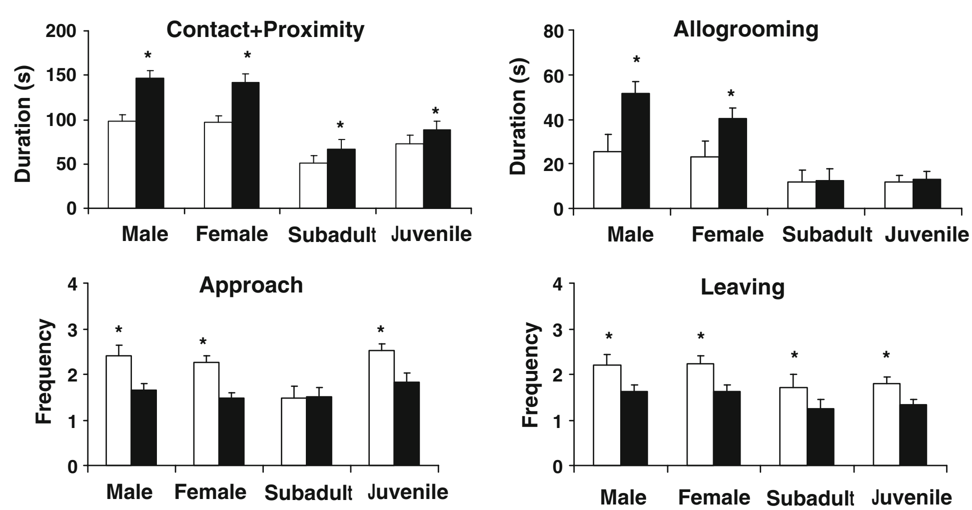

Fig. 1 Mean time (±SEM) spent in affiliative behaviors by adult males ($N = 5$), adult females ($N = 5$), subadults ($N = 2$), and juveniles ($N = 10$) regarding differences in management routine on weekdays (*white bars*) and weekends (*black bars*) in a captive colony. *Asterisks* indicate statistically significant differences between weekday (non-quiet) and weekend (quiet) conditions (Wilcoxon, $P \leq 0.05$)

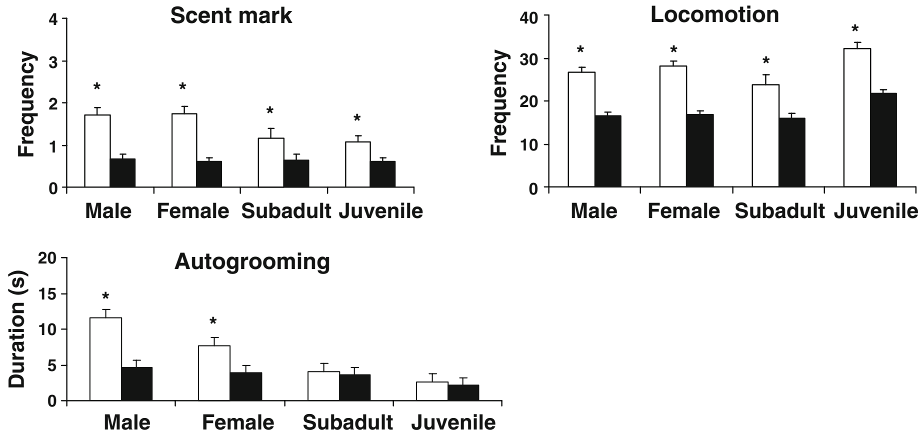

Fig. 2 Mean time (±SEM) of individual behaviors on weekdays (*white bars*) and weekends (*black bars*). *Asterisks* indicate statistically significant differences between weekday (non-quiet) and weekend (quiet) conditions (Wilcoxon, $P \leq 0.05$)

increase in individual behavior frequency on weekdays and longer affiliative behavior duration at weekends in breeding pairs. It is interesting to note that even when the social environment is similar to natural conditions, because of use of family groups (breeding pair and their offspring), the animals responded significantly to environmental changes. This assumption is supported by the increased frequency of leaving on weekdays and the interruption of social interaction. In the field, this response has also been recorded when any disturbance (e.g., the observer's approach or a distant vocalization) is perceived by the animals that are in contact/proximity or in allogrooming. In general, they look around, vocalize or even leave the partner (M. F Arruda, personal communication). In callitrichids, groups are relatively stable over time and are maintained by a complex social relationship (Rylands 1993) that could have a "buffering effect" on the behavioral pattern during exposure to disturbing conditions. This was not observed in our study.

In a *Saguinus oedipus* group, affiliative behaviors decreased during the presence of visitors in a zoo environment (Glatston et al. 1984), showing that social interaction might be disrupted by environmental disturbance. In this sense, Smith and French (1997) evaluating the behavioral pattern of heterosexual pairmates in *C. kuhli* to social deprivation found that the presence of partners reduced the behavioral response of exposure to novelty (new cage). On the other hand, individuals housed alone in the new cage spent more time in close proximity to their pairmates when returned to their home cage. These results indicated that the social partner facilitates the adjustment of behavioral response to a stressful situation. It is important to point out that, in callitrichids, the affiliative interaction between breeding pairs is related to the establishment and maintenance of pair-bond, territorial defense, and parental care (Epple 1970; Evans and Poole 1983; Soini 1987; Mota et al. 1995). Based on the role of affiliative bonding in the social and reproductive dynamics of callitrichid groups, the maintenance of a stable environment might be important for their social unit.

Moreover, all animals showed an increase in scent marking and locomotion on weekdays. In callitrichids, scent marking has been associated with the establishment and maintenance of dominance hierarchies, identification of food sources, and territorial defense (Epple 1970; Smith and Abbott 1998; Lazaro-Perea et al. 1999). Harrison and Tardif (1988) found an increase in scent marking in *S. oedipus* and *C. jacchus* males and females in the presence of intruders in their physical environment. Similarly, French and Inglett (1991) reported that *S. oedipus* females and *Leontopithecus rosalia* males scent marked more and showed an increase in agonistic behavior in the presence of non-related conspecifics. Bassett et al. (2003) found an increase in locomotion and scent marking after capturing and weighing procedures in common marmoset males and females, which may be a stressful response to these contexts. A relationship between locomotion and distress and agitation was also found by Smith et al. (1998) among animals housed in a new cage without their social partners. According to these authors, levels of locomotion may provide a noninvasive index of stress in marmosets. In our study, no direct interaction occurred between the animals and human beings; however, the higher levels of both behaviors on weekdays could be a defense reaction to disruption in their territory, despite their routine exposure to the sight and sound of humans. Even being held in captivity for a number of generations (nineteen captive-born and three wild-caught as infants), captivity did not modify their basic threatening response to the presence of intruders, which is likely to have an adaptative value for survival. Moreover, chimpanzees living in a laboratory facility had three times more episodes of wounding on weekdays, when human activity was high, than on weekends (Lambeth et al. 1997). Even so, Hosey (2000) suggests that human-animal interaction and chronic exposure to human visitors may be good for the animal, because it might reduce the stressful effect of visitation.

Breeding pairs groomed conspecifics and were groomed more often at weekends, a behavior that has been related to affiliative bonding of a group (Rothe 1974; Kleiman 1978; Goldeizen 1989). Silva and Sousa (1997) suggested allogrooming was part of male common marmosets reproductive strategy whereas Schino et al. (1988) described it as a tension-reduction mechanism. Moreover, *Macaca nemestrina* females living in social groups showed a variation in heart rate after agonistic interaction when allo or autogrooming was not recorded (Boccia et al. 1989). As found for Old World primates, the increase in autogrooming in common marmosets on weekdays reinforces the assumption of its effect as a relaxing strategy for reducing tension related to a stressful context. Further, juvenile grooming pattern was not influenced by management routine, a fact which may be related to their age. In this sense, Simonds (1974) and Goosen (1987) point out that adult non-human primates spend more time on these activities than do younger animals. A direct relationship was found between allogrooming and age in *C. jacchus*, with adults grooming the other members of social group more than they did juveniles, which in turn groomed more than infants (Woodcock 1978).

Regarding the hormonal response, despite no significance difference being found between the tested conditions, all members of the family groups showed an increase in cortisol levels during the weekdays (non-quiet) related to changes in husbandry in the colony. This finding is not in agreement with studies using callitrichids to demonstrate the role of social partners to minimize the hormonal response to stressors. In this sense, Smith and French (1997) found an elevation in cortisol levels in male and female of *C. khuli* when socially isolated from their group. Moreover, Smith et al. (1998) reported an increase in cortisol levels in response to environmental change (new cage); however, the degree of increase was less if they were with their partner, suggesting that social bonds might minimize hypothalamus–pituitary–adrenal (HPA) axis activity. In our study it seems one result of living in a stable social group is that an intense hormonal response was not observed on weekdays, probably related to a "buffering effect". This might explain the weak variation in cortisol levels despite the exposure to an environmentally stressful situation. Additional studies must be developed to evaluate the effect of social unit on the cortisol levels by housing each family member individually and evaluating their behavioral and physiological response to the same environment changes.

In our laboratory, Silva (2003) observed an increase in fecal cortisol levels in juvenile and subadult males and females of *C. jacchus* during social isolation from their family group. More recently, Galvão-Coelho et al. (2008) evaluated the cortisol levels of isosexual and heterosexual dyads of common marmoset to social changes. According to their results, the main determinant of hormonal response to social deprivation was the animals' cortisol levels before separation. Animals with low cortisol levels in the basal phase were significantly more reactive to separation. A similar hormonal response to partner separation was found in *C. jacchus* (Norcross and Newman 1999), *S. oedipus* (Ziegler et al. 1995), and *Callicebus moloch* (Mendoza and Mason 1989). Similarly, Cross et al. (2004) reported an increase in HPA axis response in adult males and females of common marmosets exposed to two different maintenance conditions. They observed a fourfold increase in salivary cortisol levels during an environmental disturbance (noise) period compared to those in a quiet period. The disturbance was caused by increased human activity and loud sounds in the colony facilities where they were housed. Thus, this acute hormonal response exhibited by males and females may be a form of maintaining their homeostatic state.

In conclusion, the results presented here suggest that the behavioral and hormonal responses of marmosets are influenced by routine management tasks even in individuals living in captivity for long period of time. The presence of social partners (pairmate and relative) reduced the physiological response, but not the behavioral response, of common marmosets. Thus, it is important to adjust husbandry in order to avoid disturbance of social interactions, individual behaviors, and hormonal profiles in captivity, as found in this study for breeding pairs and older offspring in *Callithrix jacchus*.

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