

Do newborn vocalizations affect the behavioral and hormonal responses of nonreproductive male common marmosets (*Callithrix jacchus*)?

Maricele Nascimento Barbosa ·
Maria Teresa da Silva Mota

Received: 30 July 2012 / Accepted: 24 December 2013 / Published online: 7 January 2014
© Japan Monkey Centre and Springer Japan 2014

Abstract Parental care in mammals is influenced by sensory stimuli from infants, such as sight and sound, and by changes in the hormone levels of caretakers. To determine the responsiveness of common marmoset (*Callithrix jacchus*) adult males with and without previous experience in caretaking to newborn sensory cues, we exposed twelve males to infant vocalization recordings and assessed their hormonal and behavioral responses. Males were placed in the testing cage for 10 min under two conditions: (a) control condition (exposure to adult conspecific vocalization recordings), and (b) experimental condition (exposure to infant vocalization recordings). We recorded the frequency of approach towards the sound source, the time spent near it and locomotion frequency of males in the cage under both conditions. Blood samples were collected after each test for cortisol, measured by the enzyme immunoassay method. Infant vocalization affects the behavioral and hormonal responses of non-reproductive male common marmosets. All males approached and spent more time near the sound source and showed an increase in locomotion during infant vocalization exposure compared to the control condition. Successive exposure to infant vocalization increased the responsiveness in inexperienced males. Cortisol levels were significantly higher following infant vocalization exposure compared to the control condition. These findings support the assumption that sound stimuli from the newborn are critical in initiating and maintaining caretaker responsiveness and that cortisol seems to be

important for alertness to sensory stimuli, modulating their motivation to interact with infants.

Keywords Alloparental care · Infant vocalizations · Cortisol · Previous experience · Marmosets · *Callithrix jacchus*

Introduction

Caretaking by one or both parents is critical for the survival and development of infants in most vertebrate species (Trivers 1972). This behavioral pattern can be classified as direct (cleaning, grooming, feeding, carrying) and indirect (nest building, territorial defense) (Snowdon and Soini 1982; Clutton-Brock 1991) and can be exhibited by one or both parents. Among mammals, paternal care is relatively uncommon and has been observed in carnivores, rodents, and primates (Kleiman and Malcolm 1981; Moehlman and Hofer 1997; Solomon and Getz 1997).

In non-human primates such as *Aotus trivirgatus* (Dixon and Fleming 1981) and *Callicebus moloch* (Hoffman et al. 1995), males participate in infant carrying, food sharing, and their socialization. Similarly, in marmosets and tamarins, small New World primates belonging to the Callitrichidae family (Goldizen 1987), reproductive males in the social group show extensive parental care. Moreover, the older offspring living with their social group also participate in infant carrying, which characterizes alloparental care (Tardif 1997; Bales et al. 2000).

Callitrichid groups are formed by a breeding pair, their offspring and non-related individuals (Ferrari and Digby 1996). They are described as cooperative breeders, with all members of the social unit participating in infant care, (Yamamoto 1993; Tardif 1997; Washabaugh et al. 2002),

M. N. Barbosa (✉) · M. T. S. Mota
Departamento de Fisiologia, Centro de Biociências,
Universidade Federal do Rio Grande do Norte, Campus
Universitário, Caixa Postal 1511, Natal, RN 59078-970, Brazil
e-mail: maricelebarbosa@yahoo.com.br

which is related to the costs of carrying behavior, particularly for adult females regarding the high litter—maternal weight ratio at birth, post-partum estrus (Epple 1970; Dixson and Lunn 1987; Ziegler et al. 1996), and lactation. By taking part in caretaking, animals may have several benefits, such as inclusive fitness, by investing in a relative, breeding status acquisition, and learning parental skills (Emlen 1991; Price 1992; Snowdon 1996; Bales et al. 2000).

The strongest factor that influences caretaking is previous caretaker experience. In *Macaca mulata* and *Callicebus moloch*, experienced animals retrieved infants quicker than did inexperienced ones (Gibber and Goy 1985; Hoffman et al. 1995). In callitrichids, Snowdon (1996) showed a decrease in the parental success of male and female cotton-top tamarin (*Saguinus oedipus*) without previous parenting experience. Additionally, Ingram (1977) and Pryce (1993) found an increase in infant carrying participation in experienced caretakers in *Callithrix jacchus*. Experienced mothers were more responsive to infants and showed an increase in infant survival rate.

Further evidence of caretaker responsiveness comes from a process called sensitization (Rosenblatt 1967), where the animals are continuously exposed to infants. In mammals, the somatosensory stimuli of infants such as sight, scent, and sound can modify the behavioral and hormonal components of parental care in males and females (Rosenblatt et al. 1979; Poindron and Le Neindre 1980; Fleming et al. 1997). From this perspective, the experience that animals acquire from interacting with related newborns has important long-term consequences, such as reduced latency in the display of parental behavior toward their own offspring (Fleming and Sarker 1990) and decrease in the occurrence of aversive behaviors, such as rejection or infanticide, as observed in male rodents (Elwood and Kennedy 1991; Huck et al. 1982). Thus, the behavioral response of reproductively naive caregivers is modulated by exposure to unknown offspring, which seems to be related to the recognition of sensory cues from newborns.

Some studies have investigated the male motivation to care for infants in marmosets (Roberts et al. 2001a; Ziegler et al. 2009; Saito et al. 2011). Roberts et al. (2001a) noted an increase in the rate of recovery, from 29 % on the first day of exposure to 59 % by the fourth day of exposure to infants, for inexperienced male marmosets. In addition, Saito et al. (2011) applied an infant-retrieval test in which an infant is presented to the subject to evaluate their motivation to care. Older siblings took a significantly longer time to retrieve infants than fathers during the first few days, but their latency became the same as that of parents in the 8-day test period. In both studies, successive exposure to sensory stimuli from newborns appeared to play a role in sensitizing males to caregiving behavior.

With respect to sound stimulation, non-reproductive male responsiveness to infant vocalization and infant cues as a possible releaser of caretaking or other solicitous behavior from parents was not well investigated. However, in *C. jacchus*, Zahed et al. (2008) tested the fathers and non-father responsiveness to infant vocalizations. Fathers were significantly more responsive to the unfamiliar infants and infant vocalizations than the inexperienced males. Additionally, experienced fathers showed significantly shorter latencies in entering the stimulus cage than the inexperienced males. Thus, experience has also been identified as an important factor regulating parental caregiving behavior among callitrichid primates. In females, some authors evaluated the behavioral response of female non-human primates to the vocalization of their own infants and also to unrelated individuals to determine whether they could distinguish between them (Kaplan et al. 1978; Jovanic et al. 2000; Shizawa et al. 2005). For instance, female squirrel monkeys (*Saimiri sciureus*; Symmes and Biben 1985) and Barbary macaques (*Macaca sylvanus*; Hammerschmidt and Fischer 1998) that recognized their infant call spent more time near the sensory stimuli when visual contact was constrained. Pryce et al. (1993) used an operant conditioning paradigm to train adult female marmosets to press a bar in order to simultaneously (1) gain visual access to a replica of an infant marmoset and (2) turn off an audio tape recording of infant distress vocalizations. Rates of bar pressing by first-time pregnant females were higher during late pregnancy and the early postpartum period than during early to mid-pregnancy. The authors suggest hormonal changes occurring during late pregnancy increase maternal responsiveness in common marmosets.

Infant stimuli also promote changes in the hormonal response of caregivers in callitrichids. Prolactin effects on parenting behavior have been examined both within the family and outside of the family context. Dixson and George (1982) demonstrated that high levels of prolactin were associated with carrying by fathers. Mota et al. (2006) and Mota and Sousa (2000) also found that prolactin levels increased after carrying infants in both fathers and older offspring. In other study, the influence of prolactin on male motivation was investigated in parentally inexperienced common marmosets using a prolactin antagonist (bromocriptine). Bromocriptine treatment eliminated infant retrieval in four of the eight marmosets and was associated with significantly increased retrieval latencies and significantly reduced carrying durations in the four monkeys that continued to retrieve following bromocriptine treatment (Roberts et al. 2001b). In humans, prolactin levels of fathers increase close to the birth of infants and remain high after parturition (Storey et al. 2000). Moreover, high prolactin levels were associated with responsiveness to infant cries (Fleming et al. 2002).

Mammalian hormones such as cortisol and corticosterone have been related to parental care (humans: Fleming et al. 1997; Storey et al. 2000; rats: Rees et al. 2004) and the social bond between breeding pairs (rodents: Carter 1988; Carter and Altemuss 1997). In humans, mothers with high levels of cortisol during the postpartum period physically interact more with their baby, and are more responsive to their crying and odors (Fleming and Anderson 1987; Fleming et al. 1997; Stallings et al. 2001). In meerkats (*Suricata suricatta*), males showed an increase in cortisol during infant care (Carlson et al. 2006), whereas in callitrichids cortisol levels were either reduced (*Callithrix kuhlii*: Nunes et al. 2001) or unchanged (*Saguinus oedipus*: Ziegler et al. 1996). However, the role of cortisol in parental care in males is poorly understood.

In family groups, parents and siblings are highly interested in newborns and infants and seem to compete for infant care (Yamamoto and Box 1997). In this scenario, male responsiveness to infants may be affected by the social environment. Therefore, the aims of this study were: (a) to evaluate whether the hormonal (cortisol) and behavioral responses change in non-reproductive adult male common marmosets (*Callithrix jacchus*) when exposed to infant vocalization repeatedly, and (b) to determine if previous experience in alloparental care influenced the hormonal levels and behavioral response of males when exposed to infant vocalization.

Methods

Animals

Twelve non-reproductive adult male common marmosets (*Callithrix jacchus*), were used. The males ranged in age from 21 to 48 months at the time of testing. All animals were captive-born and 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). All animals were categorized as either experienced or inexperienced in alloparental care, based on the number of younger siblings with which they lived previously. Six males were experienced in alloparental care: four of them experienced with 1 set of infants and two others with 2 sets (mean \pm SEM: 3 ± 1 sets of younger siblings). The other six males had no previous experience in alloparental care. The age of the experienced males ranged from 32 to 48 months while the inexperienced ones ranged from 21 to 38 months. No difference was found in age between the experienced and inexperienced groups (independent *t*-test: $t = 1.98$, $P = 0.56$).

They were previously housed with their family groups and 3 months before the experiment start, males were

removed from their cages and individually housed. Each animal stayed in wire mesh and brick wall outdoor cages measuring $2.00 \times 1.00 \times 2.00$ m under natural light–dark cycles. The animals in the same colony room were denied visual contact but retained olfactory and auditory contact with neighboring enclosures. The animals were fed twice a day around 9:00 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. Each animal also received an iron and vitamin complex supplement (2 ml) mixed in milk after blood samples were collected. The supplement was administered orally.

Experimental procedure

This study compared the behavioral pattern and hormonal profile of animals under two conditions: (a) control condition (exposure to unrelated adult animal vocalization of the same species) and (b) experimental condition (exposure to unrelated infant vocalization recordings).

Vocalization recording

To obtain the infant stimuli, 15- to 22-day-old infants ($n = 4$) were temporarily removed from their family group on a carrier for brief intervals of 15–20 min. Each infant was placed into an wire mesh cage ($1.00 \times 0.68 \times 1.00$ m) located in a separate room with no auditory, visual or olfactory cues from other marmosets. We recorded the infant cry calls (*phee* calls) produced by four male infants. *Phee* calls are high-pitched distress calls, produced in a clear social context (isolation) and are easily elicited (Miller and Wang 2006). After recording, the infant was then returned to the carrier in the nest box and returned to the family group. To obtain the adult vocalization recordings, 30- to 40-month-old males ($n = 4$) were temporarily removed from their home cage and placed in the test cage ($1.00 \times 0.68 \times 1.00$ m) located in the same room that was used to record infant vocalizations. We recorded the *phee* calls produced by an isolated animal in the test cage. After recording, the animals returned to their home cages. The vocalizations of infants and adults were collected with a professional recorder (Marantz PMD 30) connected to a Sennheiser (Sennheiser ME 80) (frequency range 50–15,000 Hz) unidirectional microphone. There was no reverberation in the recording environment. The recordings were conducted at a distance of 0.5 m from the subjects. Prior to each recording session, animals were allowed to habituate to the presence of an observer and recording equipment.

Table 1 Behavioral categories recorded

Behavior	Definition
Locomotion	The cage was divided into 22 quadrants of 23 cm each and the frequency of movement by the focal animal between two quadrants was recorded as 1 movement
Scent marking	Frequency of rubbing the anogenital region on a cage surface
Approach and leaving	Frequency of going towards and away from the sound source from an imaginary boundary of 50 cm for more than 3 s
Proximity	Time spent within 50 cm of the sound source for more than 3 s

Vocal stimuli response tests

On test days, subjects were removed from their home cages in their nest box and placed in a test cage (1.00 × 0.68 × 1.00 m) located in a soundproof room with no auditory, visual or olfactory cues from other marmosets of the colony, except the specific stimuli provided for the test. They were habituated to the test environment for 10 days. To determine the responsiveness of non-reproductive males to sensory stimulation, adult and infant vocalizations were played back using a portable recorder (Philips MCM148/55) located approximately 0.5 m from test cage and covered with a cloth to prevent animal visualization. The sound pressure level of stimulus presentation was 50 dB SPL. The males had no experience of hearing the voice of stimulus animals.

Each animal was exposed to infant and adult vocalization recordings twice a week (Tuesday and Friday), over a period of 4 consecutive weeks, with a total of four tests for each subject in each condition. Thus, each male was exposed 4 times to infant vocalization and adult vocalization recordings. However, each male was presented to four infant stimuli, so each stimulus was presented once. In a 10-min interval, the stimulus was presented 4 times for 1 min with a 2-min interval between them, totaling 4 min of vocalization recording exposure. The order of stimulus presentation (whether the “vocalization” was an infant or adult) was alternated. Behavioral observations were conducted during the tests for 10 min between 10:00 and 11:30 am by continuous focal sampling, and the behavioral variables recorded are shown in Table 1. All the observation sessions were made through a one-way mirror window by one observer and the behavioral data were collected on check sheets.

Blood collection

Blood sample collection was carried out twice a week starting 2 weeks before the test to evaluate basal cortisol levels. During the tests, blood samples were collected

immediately after the behavioral observation in both conditions (control and experimental) to evaluate the cortisol response of male common marmoset to vocal stimulus. All blood samples (0.5 ml) were collected by femoral venipuncture. A 1-ml heparinized syringe (Liquemine, Roche Pharmacy Laboratory) was attached to a 27.5G needle. Before blood collection, the subjects were habituated to the capture and handling procedure in order to diminish their stress responsiveness to the restraint situation. The animals were held using a gloved hand and were removed from the cage twice a week. They were placed in a restraint device for small primates (Hearn 1983), and blood samples were obtained. Using this procedure, it was possible to have blood samples within 80–150 s after handling the subjects. Following each procedure, the animals received an iron supplement and additional vitamins and were rewarded with sweet milk. After this, samples were then centrifuged for 10 min at 3,000 rpm and the plasma was stored at −20 °C.

Cortisol assay

The analysis was run at the Hormonal Measures Laboratory in the Physiology Department of the Universidade do Rio Grande do Norte, Natal-RN, Brazil. Cortisol plasma levels were determined using an enzyme immunoassay (EIA). The technique was performed according to the protocol developed by Munro and Stabenfeldt (1984) and modified by the Wisconsin Regional Primate Research CTR. Intra-assay and inter-assay coefficients of variation were 1.56 and 5.49 % for low pool and 1.57 and 3.54 % for high pool, respectively. Serial dilutions of marmoset-pooled plasma were used to demonstrate parallelism (no difference between slopes, $t = -1.50$, $P > 0.05$) and accuracy was 91.7 ± 5.4 %, $n = 12$.

Statistical analyses

Before statistical analysis was carried out, both behavioral and hormonal data were evaluated with respect to their normal distribution, and a log transformation was performed for behavioral data. Parametric statistical analyses were conducted. To evaluate the effect of conditions (infant or adult vocalization) and previous experience (experienced vs inexperienced) on the behavioral data of non-reproductive marmoset males, a two-way analysis of variance (ANOVA) with post hoc Tukey test was used. Cortisol levels were evaluated by ANOVA with post hoc Tukey test using testing conditions (basal vs infant vocalization vs adult vocalization) as within group factors and experience (experienced vs inexperienced) as between group factors. Additionally, the effect of successive exposures to vocal stimuli (exposure days) on the behavioral and hormonal data of non-reproductive marmosets was analyzed using an ANOVA (post hoc

Tukey test). The Pearson correlation test was used to evaluate the relationship among behavioral variables and plasma cortisol levels. P values ≤ 0.05 were considered significant.

Results

The behavioral pattern of non-reproductive adult males was significantly influenced by exposure to infant vocalization. A comparison between both conditions (infant or adult vocalization) revealed that all males approached and spent more time near the sound source and showed an increase in locomotion during infant sound exposure compared to the control condition (ANOVA: approach $F_{1,46} = 18.62$, $P = 0.002$; proximity time $F_{1,46} = 16.98$, $P = 0.004$; locomotion $F_{1,46} = 12.11$, $P = 0.008$) (Fig. 1a, b). No difference was found for leaving and scent marking frequency, respectively (ANOVA: $F_{1,46} = 2.21$, $P = 0.123$; $F_{1,46} = 1.32$, $P = 0.225$).

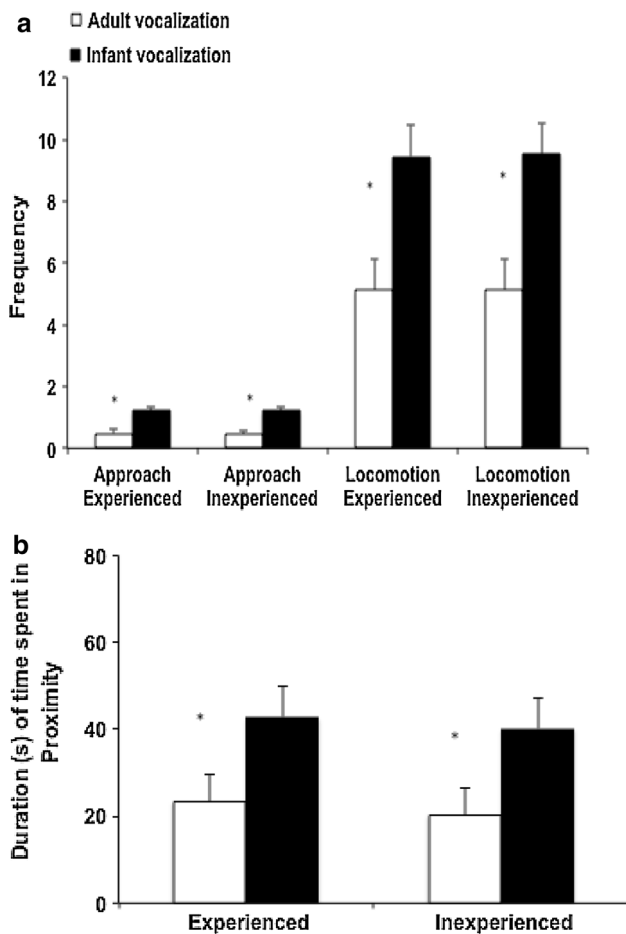


Fig. 1 Frequency (a) and mean time (b) ($X \pm SEM$) of behaviors exhibited by nonbreeding adult male marmoset, *Callithrix jacchus*, when exposed to adult vocalization (white bar) and infant vocalization (black bar). Asterisks indicate statistical difference, $P \leq 0.05$ (ANOVA, $P \leq 0.05$)

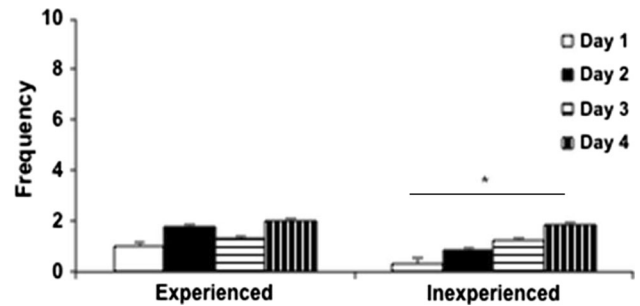


Fig. 2 Frequency ($X \pm SEM$) of approaches exhibited by experienced and inexperienced adult male marmosets, *Callithrix jacchus*, during days of exposure to infant vocalizations. Asterisks indicate statistically significant differences between the 4th day of exposure to infant vocalizations, compared to the 1st day (repeated-measures ANOVA, $P \leq 0.05$)

No interaction was found between experimental condition and previous experience (ANOVA: locomotion $F_{1,46} = 1.51$, $P = 0.162$; approaches $F_{1,46} = 1.98$, $P = 0.902$; scent mark $F_{1,46} = 1.46$, $P = 0.234$; leaving $F_{1,46} = 1.76$, $P = 0.786$; proximity time $F_{1,46} = 0.78$, $P = 0.392$).

Evaluating the effect of successive exposure (days) to infant vocalization on the behavioral response of males, significant difference was found for frequency of approaches to the sound source only in inexperienced males (ANOVA: $F_{3,30} = 5.50$, $P = 0.038$) (Fig. 2). We found a significant interaction between days of exposure to infant vocalization and prior experience for frequency of approaches (ANOVA: $F_{3,30} = 4.50$, $P = 0.041$). The inexperienced males approached more frequently on the 4th day of exposure to infant vocalization, compared to the 1st day (ANOVA: $F_{3,30} = 3.20$, $P = 0.024$; Fig. 2). The other observed behaviors did not vary with successive days of exposure to the newborns (ANOVA: scent marking $F_{3,30} = 1.50$, $P = 0.256$, proximity time $F_{3,30} = 2.65$, $P = 0.081$, locomotion $F_{3,30} = 2.50$, $P = 0.095$, leaving $F_{3,30} = 2.70$, $P = 0.095$). No interaction was found between days of exposure to infant vocalization and previous experience for leaving (ANOVA: $F_{3,30} = 1.10$, $P = 0.332$), scent marking ($F_{3,30} = 1.55$, $P = 0.225$), proximity time ($F_{3,30} = 2.57$, $P = 0.253$) and locomotion ($F_{3,30} = 1.28$, $P = 0.093$). No difference was found in the behavioral response of males between days of exposure to adult vocalization (control condition) (ANOVA: approaches $F_{3,30} = 1.70$, $P = 0.353$; scent marking $F_{3,30} = 1.67$, $P = 0.201$, proximity time $F_{3,30} = 2.75$, $P = 0.11$, locomotion $F_{3,30} = 2.40$, $P = 0.095$, leaving $F_{3,30} = 2.60$, $P = 0.45$).

Hormonal response

The comparison of the mean cortisol levels of males between the conditions tested (basal vs adult vocalization

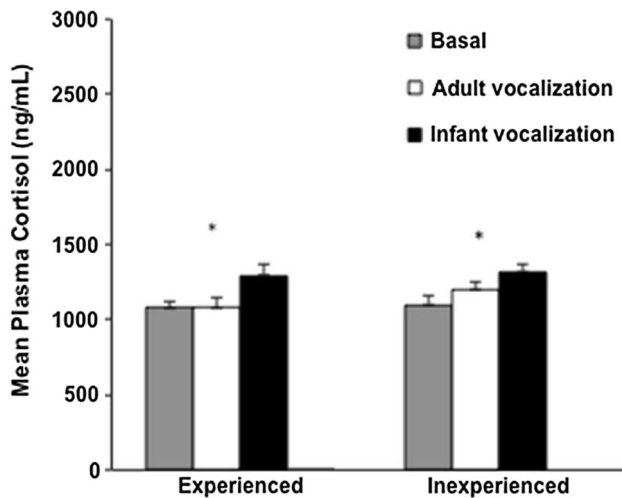


Fig. 3 Plasma cortisol levels (mean \pm SE) of experienced and inexperienced males in the basal condition (gray bar) and when exposed to adult vocalizations (white bar) and to infant vocalization recordings (black bar). Asterisks indicate statistical difference, $P \leq 0.05$ (dependent t test)

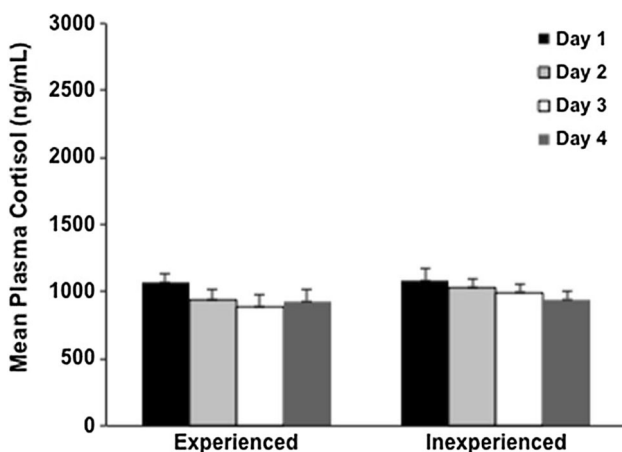


Fig. 4 Plasma cortisol levels (ng/ml) (mean \pm SE) of experienced and inexperienced adult male marmosets, *C. jacchus*, over days of exposure to infant vocalization (repeated-measures ANOVA, $P \leq 0.05$)

vs infant vocalization) showed that highest cortisol levels were found after infant vocalization presentation (ANOVA: $F_{2,92} = 2.86$, $P = 0.041$, post hoc Tukey test, $P < 0.050$; Fig. 3). No interaction was found for plasma cortisol between the conditions and previous experience (ANOVA: $F_{2,92} = 2.25$, $P = 0.111$). No significant difference in cortisol levels was found for experienced males (ANOVA: $F_{3,30} = 1.55$, $P = 0.091$) and inexperienced males (ANOVA: $F_{3,30} = 1.93$, $P = 0.104$) between days of exposure to newborns (Fig. 4). In addition, no interaction was found for plasma cortisol between the exposure days and previous experience (ANOVA: $F_{2,92} = 2.25$,

$P = 0.132$). There was also no correlation between behavioral variables and plasma cortisol levels in adult males (Pearson correlation test: locomotion $r = 0.21$; $P = 0.256$; approaches $r = 0.30$; $P = 0.205$; scent mark $r = 0.18$; $P = 0.272$; proximity time $r = 0.39$; $P = 0.10$; leaving $r = 0.11$; $P = 0.306$).

Discussion

It can be concluded that infant vocalization affects the behavioral and hormonal responses of non-reproductive male common marmosets. The increase in approach frequency, time spent near the sound source and locomotion during sound presentation suggest an interest and motivation on the part of males to interact with the infant. These findings support the assumption that sensory cues from newborn are critical in initiating and maintaining caretaker responsiveness.

The use of a vocal stimulus compared to a live infant has many advantages. For example, removing an infant from its family and using it as a stimulus could be stressful to the infant (Pryce et al. 2002). In addition, vocal recordings can be used repeatedly, serving as novel stimuli for multiple males. Our study showed that males looked under the portable recorder attempting to find the “infant”. These behaviors indicate that recorded vocal stimuli of an infant are as salient a signal as the live infant.

Infant stimuli, particularly crying, are considered to be distress signals that originally evolved, along with other attachment behaviors, to promote proximity to or contact between infants and their caregivers (Kaplan et al. 1978). Thus, close proximity between the caregiver and infant plays a role in infant protection against predators in a dangerous environment. Thus, the vocalization emitted by an infant when it loses sight of its parents is useful for estimating infant requirements for parental care. Moreover, the parental responsiveness to signals from infants is important for understanding the proximate regulation of the parent–infant relationship and their evolutionary dynamics.

In this sense, infant vocalization usually communicates an affective state and leads to an immediate response from the caretaker (Kaplan et al. 1978). For example, Elowson et al. (1998) showed that pygmy marmoset (*Callithrix pygmaea*) caretakers approached and picked up infants more when they were vocalizing (babbling) than when they were not. Jovanic et al. (2000) used a playback experiment to demonstrate that rhesus macaque (*Macaca mulatta*) mothers were responsive to infant vocalization and were able to recognize their own infant’s call. A similar response was obtained in Japanese macaque (*Macaca fuscata*) mothers by Shizawa et al. (2005). These findings support the assumption that vocal stimulus is important for

caretaking behavior elicitation in mothers and other caretakers such as the non-reproductive male common marmoset used in our study.

Another important point concerning caretaking behavior is previous infant care experience. Data from Epple (1975) showed a decrease in survival of the first set of infants in inexperienced female saddle-back tamarins (*Saguinus fuscicollis*). According to the author, an increase in motivation to carry and the type of social environment experienced by animals during development may be as important as direct infant carrying experience in learning parental skills. A similar trend was found in female cotton-top tamarins, but not in marmosets (Tardif et al. 1984). With respect to non-reproductive animals, no difference was found in carrying bout duration and retrieval frequency between subadult cotton-top tamarins previously exposed to infants and inexperienced individuals (Tardif et al. 1992). Some studies have shown that experienced fathers spend more time carrying their infants than do first-time fathers (*Callithrix kuhli*: Nunes et al. 2001; *Saguinus oedipus*: Ziegler et al. 2000, 2004). In addition, experienced adult male marmosets exhibit a greater frequency of infant-directed behavior when compared with inexperienced males in response to unfamiliar infant vocalizations (infant distress calls) (*Callithrix jacchus*: Zahed et al. 2008). Our study showed that non-reproductive males, regardless of previous experience in infant care, showed a strong behavioral response to infant vocalization by increasing stimuli-directed behaviors (i.e. approaching, and staying close to the sound source). It seems that caretaker exposure to infants while living with their family group as a sibling or parent is important for positive reinforcement of parental skills acquisition. Accordingly, vocalization might be related to hunger, pain, decreased body temperature or separation from the mother or another caretaker. Thus, in cooperative breeding systems, all individuals of a social unit are responsive to infant vocalization, a behavior that may be a critical adaptation for infant survival, especially in the field where the predation risk is much higher than in captivity.

In humans, fathers exposed to an infant cry recording showed a strong and immediate response to related and unrelated infant cries compared to inexperienced fathers. Experienced fathers exposed to sound stimulus were more sympathetic and alert (Fleming et al. 2002). Common marmoset males were very responsive to newborn vocal stimuli, a behavior that may be related to maintaining proximity and the caretaker–infant interaction during early development. Despite the influence of previous experience on the behavioral pattern of caretakers in callitrichids and other primate species, in the present study no response difference was found between experienced and non-experienced individuals to infant vocalization. Males were

interested and motivated to socially interact with the infant stimuli, regardless of caretaking experience, suggesting that experience is important but not essential to caretaker responsiveness to infant vocalization in common marmosets.

However, we observed a change in the responsiveness of inexperienced males over successive days of exposure to infant vocalization. The inexperienced males approached more frequently on the 4th day of exposure to infant vocalization, compared to the 1st day. In a recent study, we observed a change in the responsiveness of inexperienced males over successive days of exposure to newborns. Inexperienced males carried newborns for longer periods on the 4th day of exposure, compared to the 1st day (Barbosa and Mota 2013). In both studies, successive exposure to sensory stimuli from newborns appeared to play a role in sensitizing inexperienced males to caregiving behavior. According to Rosenblatt (1967), continued exposure to the sensory cues of newborns would be the main element in triggering parental behavior, which is not modulated by circulating hormones.

Regarding the hormonal profile of males in the current study, we found an effect of sensory stimulus on cortisol in response to infant vocalization exposure. Cortisol levels were significantly higher following infant vocalization exposure compared to the control condition. These findings support the assumption that sound stimuli from newborn are critical in initiating and maintaining caretaker responsiveness and that cortisol seems to be important for alertness to sensory stimuli, modulating their motivation to interact with infants. However, the marmosets exhibited larger concentrations of cortisol, although not significant, when individuals were exposed to vocalizations from unfamiliar conspecifics. Rukstalis and French (2005) demonstrated that marmosets removed from their home cage and exposed to unfamiliar marmoset vocalizations showed significantly higher levels of urinary cortisol than when exposed to a familiar pair mate's vocalization or to no auditory stimuli. In our study, cortisol levels were influenced by infant vocalization, suggesting an increased vigilant state that may be important in affiliative interaction.

In a previous study, we observed an increase in plasma cortisol levels after exposure to newborns in sealed enclosures when compared to open box and to control conditions (without an infant) (Barbosa and Mota 2013). Given that under this experimental condition adult males could only see the newborn and not touch or smell it, the increased hormonal response suggests a physiological reaction to the stress of experiencing restricted access to the newborn. However, Fleming (1993) found an increase in cortisol levels after women were exposed to the body odor of their infants. Mothers with the highest cortisol

levels were also better able to recognize their infants' body odors. Corroborating these findings, Carter (1988) and Fleming et al. (1993) associated cortisol with mother–infant attachment and social bond formation (Carter 1988), respectively. This finding suggests that cortisol may be related to a state of alertness, causing males to be more aware and more responsive to infant cues. In marmosets, both mothers and fathers show hormonal changes during pregnancy, after parturition of their mates, and after smelling infant odors (Torii et al. 1998; Nunes et al. 2001; Schradin and Anzenberger 2004; Ziegler et al. 2011). Prudom et al. (2008) found that fathers exposed to isolated scents of their infant displayed a significant drop in serum testosterone levels within 20 min after exposure, whereas parentally naive males did not. In addition, fathers with decreased testosterone have a greater need to respond to infant stimuli compared with non-fathers or males who did not have offspring of their own, when tested with infant cries (Fleming et al. 2002).

High circulating cortisol levels appear to enhance arousal and responsiveness to infant stimuli in inexperienced female primates, but interfere with the expression of maternal behavior in experienced females (Bardi et al. 2003; Saltzman and Maestripieri 2011). In free-ranging yellow baboons (*Papio cynocephalus*), inexperienced females with high fecal glucocorticoid levels were subsequently more responsive to their infants' distress cries, during the first 8 weeks post-partum, than those with lower glucocorticoid levels (Nguyen et al. 2008). In contrast, high cortisol levels are associated with diminished maternal behavior in experienced female marmosets (Saltzman and Abbott 2009). In our study, cortisol levels of experienced and inexperienced males did not change between days of exposure to infants, nor were they associated with responsiveness to infants. The values reported in our study were moderate relative to those reported in other common marmoset studies (Mota et al. 2006; Nunes et al. 2001). Although our understanding of the neuroendocrine correlates of maternal primates has grown in the last two decades, very little is known about the factors that influence the responsiveness of males to infant stimuli.

In conclusion, we found that the exposure of non-reproductive males to infant vocalization recordings seems to be an effective tool for evaluating their behavioral and hormonal response to somatosensory cues from newborns. Males behaved positively towards infant vocal signaling, which may affect their motivational state to interact with the stimulus. The association between behavioral response and cortisol levels suggests that cortisol may lead to an alert state, important for recognizing somatosensory information during infant–caretaker interaction. Future studies would do well to compare three conditions (adult vocalization, infant vocalization and a neutral one) since the exposure to

adult vocalization can be interpreted as a threat. Thus, further investigation into the role of infant vocalization and its role in regulating parental hormones is needed.

Acknowledgments We thank Mr. Antonio Barbosa da Silva and MSc José Flávio Vidal Coutinho for assistance in the observations of common marmoset males. This study was supported by grants from CNPq, FINEP and UFRN. Two anonymous reviewers provided helpful comments that much improved the manuscript.

References

- Bales K, Dietz JM, Baker A, Miller K, Tardif SD (2000) Effects of allocare-givers on fitness of infants and parents in callitrichid primates. *Folia Primatol* 71:27–38
- Barbosa MN, Mota MTS (2013) Alloparental responsiveness to newborns by nonreproductive, adult male, common marmosets (*Callithrix jacchus*). *Am J Primatol* 75(2):145–152
- Bardi M, Shimizu K, Barrett GM, Borgognini-Tarli SM, Huffman MA (2003) Peripartum cortisol levels and mother–infant interactions in Japanese macaques. *Am J Phys Anthropol* 120(3):298–304
- Carlson AA, Manser MB, Young AJ, Russel AF, Jordan NR, McNeilly AS, Clutton-Brock T (2006) Cortisol levels are positively associated with pup-feeding rates in male meerkats. *Proc R Soc B* 273:571–577
- Carter CS (1988) Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23:779–818
- Carter CS, Altemus M (1997) Integrative functions of lactational hormones in social behavior and stress management. *Ann N Y Acad Sci* 7:164–174
- Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton
- Dixson AF, Fleming D (1981) Parental behaviour and infant development in owl monkeys (*Aotus trivirgatus*). *J Zool (London)* 194:25–39
- Dixson AF, George L (1982) Prolactin and parental behaviour in a male New World primate. *Nature* 29:551–553
- Dixson AF, Lunn SF (1987) Post-partum changes in hormones and sexual behavior in captive groups of marmosets (*Callithrix jacchus*). *Physiol Behav* 41:577–583
- Elowson AM, Snowdon CT, Lazaro-Perea C (1998) “Babbling” and social context in infant monkeys: parallels to human infants. *Trends Cogn Sci* 2:31–37
- Elwood RW, Kennedy HF (1991) Selectivity in paternal and infanticidal responses by male mice: effects of relatedness, location, and previous sexual partners. *Behav Neural Biol* 56:129–147
- Emlen ST (1991) Predicting family dynamics in social vertebrates. In: Davies ND (ed) *Behavioural ecology*. Blackwell, Oxford, pp 228–253
- Epple G (1970) Maintenance, breeding and development of marmoset monkeys (*Callitrichidae*) in captivity. *Folia Primatol* 12:56–76
- Epple G (1975) The behavior of marmoset monkeys (*Callitrichidae*). In: Rosenblum LA (ed) *Primate behavior*. Academy Press, New York, pp 195–239
- Ferrari SF, Digby LJ (1996) Wild *Callithrix* groups: stable extended families? *Am J Primatol* 38:19–27
- Fleming AS (1993) Hormonal and experiential correlates of maternal responsiveness in human mothers. In: Krasnegor NA, Bridges RS (eds) *Mammalian parenting: biochemical, neurobiological, and behavioral determinants*. Oxford University Press, New York, pp 184–208

- Fleming AS, Anderson V (1987) Affect and nurturance: mechanisms mediating maternal behavior in two female mammals. *Prog Neuropsychopharmacol Biol Psychiatry* 11:121–127
- Fleming AS, Sarker J (1990) Experience-hormone interactions and maternal behavior in rats. *Physiol Behav* 47:1165–1173
- Fleming AS, Corter C, Franks P, Surbey M, Schneider B, Steiner M (1993) Postpartum factors related to mother's attraction to newborn infant odors. *Dev Psychol* 26:137–143
- Fleming AS, Ruble D, Wong PY (1997) Hormonal and experiential correlates of maternal responsiveness during pregnancy and puerperium in human mothers. *Horm Behav* 31:145–158
- Fleming AS, Corter C, Stallings J, Steiner M (2002) Testosterone and prolactin are associated with emotional responses to infant cries in new fathers. *Horm Behav* 42:399–413
- Gibber JR, Goy RW (1985) Infant-directed behavior in young rhesus monkeys: sex differences and effects of prenatal androgens. *Am J Primatol* 8:225–237
- Goldizen AW (1987) Tamarins and marmosets: communal care of offspring. In: Cheney DL, Seyfarth RM, Struhsaker TT, Smits BB (eds) *Primate societies*. University of Chicago Press, Chicago, pp 34–43
- Hammerschmidt K, Fischer J (1998) Maternal discrimination of offspring vocalizations in Barbary macaques (*Macaca sylvanus*). *Primates* 39:231–236
- Hearn JP (1983) The common marmoset (*Callithrix jacchus*). In: Hearn JP (ed) *Reproduction in New World primates in medical science*. MTP Press, Boston, pp 183–216
- Hoffman KA, Mendoza SP, Henessy MB, Mason WA (1995) Responses of infant titi monkeys, *Callicebus moloch*, to removal of one or both parents: evidence for paternal attachment. *Dev Psychobiol* 28:399–407
- Huck UH, Carter CS, Banks EM (1982) Natural or hormone-induced sexual and social behaviors in the female brown lemming (*Lemmus trimucronatus*). *Horm Behav* 16:199–207
- Ingram JC (1977) Interactions between parents and infants and the development of independence in common marmoset (*Callithrix jacchus*). *Anim Behav* 25:811–827
- Jovanic T, Megna NL, Mastripietri D (2000) Early maternal recognition of offspring vocalizations in rhesus macaques (*Macaca mulatta*). *Primates* 41:421–428
- Kaplan JN, Winship-Ball A, Lynne SI (1978) Maternal discrimination of infant vocalizations in squirrel monkeys. *Primates* 19:187–193
- Kleiman DG, Malcolm JR (1981) The evolution of male investment in mammals. In: Gubernick DJ, Worter PH (eds) *The parental care in mammals*. Plenum Press, New York, pp 181–190
- Miller CT, Wang X (2006) Sensory-motor interactions modulate a primate vocal behavior: antiphonal calling in common marmosets. *J Comp Physiol* 192:27–38
- Moehlman PD, Hofer H (1997) Cooperative breeding, reproductive suppression, and body mass in canids. In: Solomon NG, French JA (eds) *Cooperative breeding in mammals*. Cambridge University Press, Cambridge, pp 76–149
- Mota MTS, Sousa MBC (2000) Prolactin levels of fathers and helpers related to alloparental care in common marmosets, *Callithrix jacchus*. *Folia Primatol* 71:22–26
- Mota MTS, Franci CS, Sousa MBC (2006) Hormonal changes related to paternal and alloparental care in common marmosets (*Callithrix jacchus*). *Horm Behav* 49:293–302
- Munro C, Stabenfeldt G (1984) Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *J Endocrinol* 101:41–49
- Nguyen NGA, Gesquiere LR, Wango EO, Alberts SC, Altmann J (2008) Late pregnancy glucocorticoid levels predict responsiveness in wild baboon mothers (*Papio cynocephalus*). *Anim Behav* 75:1747–1756
- Nunes S, Fite JE, Patera KJ, French JA (2001) Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*). *Horm Behav* 39:70–82
- Poindron P, Le Neindre P (1980) Endocrine and sensory regulation of maternal behavior in the ewe. In: Rosenblatt JS, Hinde RA, Beer C, Busnel MC (eds) *Advances in the study of behavior*. Academic Press, New York, pp 75–119
- Price EC (1992) Sex and helping: reproductive strategies of breeding male and female cotton-top tamarins, *Saguinus oedipus*. *Anim Behav* 43:717–728
- Prudom SL, Broz CA, Schultz-Darken N, Ferris CT, Snowdon C, Ziegler TE (2008) Exposure to infant scent lowers serum testosterone in father common marmoset, *Callithrix jacchus*. *Biol Lett* 4:603–605
- Pryce CR (1993) The regulation of maternal behaviour in marmosets and tamarins. *Behav Process* 30:201–222
- Pryce CR, Dobeli M, Martin RD (1993) Effects of sex steroids on maternal motivation in the common marmoset (*Callithrix jacchus*): development and application of an operant system with maternal reinforcement. *J Comp Psychol* 107:99–115
- Pryce CR, Ruedi-Bettschen D, Dettling AC, Feldon J (2002) Early life stress: long-term physiological impact in rodents and primates. *News Physiol Sci* 17:150–155
- Rees SL, Panesar S, Steiner M, Fleming AS (2004) The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat. *Horm Behav* 46:411–419
- Roberts RL, Jenkins KT, Lawler T, Wegner FH, Norcross JL, Bernhards DE, Newman JD (2001a) Prolactin levels are elevated after infant carrying in parentally inexperienced common marmosets. *Physiol Behav* 72(5):713–720
- Roberts RL, Jenkins KT, Lawler T, Wegner FH, Newman JD (2001b) Bromocriptine administration lowers serum prolactin and disrupts parental responsiveness in common marmosets (*Callithrix jacchus*). *Horm Behav* 39:106–112
- Rosenblatt JS (1967) Nonhormonal basis of maternal behavior in the rat. *Science* 156:1512–1514
- Rosenblatt JS, Siegel HI, Mayer AD (1979) Progress in the study of maternal behavior in the rat: hormonal, nonhormonal, sensory, and developmental aspects. In: Rosenblatt JS, Hinde AC, Beer MC (eds) *Advances in the study of behavior*. Academic Press, New York, pp 225–311
- Rukstalis M, French JA (2005) Vocal buffering of the stress response: exposure to conspecific vocalizations moderates urinary cortisol excretion in isolated marmosets. *Horm Behav* 47:1–7
- Saito A, Izumi A, Nakamura K (2011) Fathers have higher motivation for parenting than mothers in common marmoset (*Callithrix jacchus*). *Behaviour* 148:1199–1214
- Saltzman W, Abbott DH (2009) Effects of elevated circulating cortisol concentrations on maternal behavior in common marmoset monkeys (*Callithrix jacchus*). *Psychoneuroendocrinology* 34:1222–1234
- Saltzman W, Maestripietri D (2011) The neuroendocrinology of primate maternal behavior. *Prog Neuropsychopharmacol Biol Psychiatry* 35:1192–1204
- Schradin C, Anzenberger G (2004) Development of prolactin levels in marmoset males: from adult son to first-time father. *Horm Behav* 46:670–677
- Shizawa Y, Nakamichi M, Hinobayashi T, Minami T (2005) Playback experiment to test maternal responses of Japanese macaques (*Macaca fuscata*) to their own infant's call when the infants were four to six months old. *Behav Process* 68:41–46
- Snowdon CT (1996) Infant care in cooperatively breeding species. In: Rosenblatt JS, Snowdon CT (eds) *Parental care: evolution, mechanisms, and adaptive significance*. Academic Press, San Diego, pp 643–689

- Snowdon CT, Soini SJ (1982) Parental behavior in primates. In: Mullins JA, Gage PT, Fitzgerald HE (eds) Child nurturance. Plenum Press, New York, pp 63–108
- Solomon NG, Getz LL (1997) Examination of alternative hypotheses for cooperative breeding in Rodentia. In: Solomon NG, French JA (eds) Cooperative breeding in mammals. Cambridge University Press, Cambridge, pp 199–230
- Stallings J, Fleming AS, Corter S, Worthman C, Steiner M (2001) The effects of infant cries and odors on sympathy, cortisol and autonomic responses in new mothers and non-postpartum women. *Parent Sci Pract* 1:71–100
- Storey AE, Walsh CJ, Quinon RL, Wynne-Edwards GT (2000) Hormonal correlates of paternal responsiveness in new and expectant fathers. *Evol Hum Behav* 21:79–95
- Symmes D, Biben M (1985) Maternal recognition of individual infant squirrel monkeys from isolation call playback. *Am J Primatol* 9:39–46
- Tardif SD (1997) The bioenergetic of parental behavior and the evolution of alloparental care in marmosets and tamarins. In: Solomon NG, French JA (eds) Cooperative breeding in mammals. Cambridge University Press, Cambridge, pp 11–33
- Tardif SD, Carson RL, Gangaware BL (1984) Comparison of infant care in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*). *Am J Primatol* 22:73–85
- Tardif SD, Carson RL, Gangaware BL (1992) Infant-care behavior of non-reproductive helpers in a communal-care primate, the cotton-top tamarin (*Saguinus oedipus*). *Ethology* 92:155–167
- Torii R, Moro M, Abbott DH, Nigi H (1998) Urine collection in the common marmoset (*Callithrix jacchus*) and its applicability to endocrinological studies. *Primates* 39:407–417
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell D (ed) Sexual selection and descent of man. Aldine Press, Chicago, pp 136–179
- Washabaugh KF, Snowdon CT, Ziegler TE (2002) Variations in care for cotton top tamarin, *Saguinus oedipus*, infants as a function of group size. *Anim Behav* 6:1163–1174
- Yamamoto ME (1993) From dependence to sexual maturity: the behavioural ontogeny of Callitrichidae. In: Rylands AR (ed) Marmosets and tamarins: systematics, behaviour, and ecology. Oxford University Press, Oxford, pp 235–254
- Yamamoto EM, Box HO (1997) The role of non-reproductive helpers in infant care in captive *Callithrix jacchus*. *Ethology* 103:760–771
- Zahed SR, Prudom SL, Snowdon CT, Ziegler TE (2008) Male parenting and response to infant stimuli in the common marmoset (*Callithrix jacchus*). *Am J Primatol* 1:84–92
- Ziegler TE, Wegner FH, Snowdon CT (1996) Hormonal responses to parental and nonparental conditions in male cotton-top tamarins, *Saguinus oedipus*, a New World primate. *Horm Behav* 30:287–297
- Ziegler TE, Wegner FH, Carlson AA, Lazaro-Perea C, Snowdon CT (2000) Prolactin levels during the preparturitional period in the biparental cotton-top tamarin (*Saguinus oedipus*): interactions with gender, androgen levels, and parenting. *Horm Behav* 38:111–122
- Ziegler TE, Washabaugh KF, Snowdon CT (2004) Responsiveness of expectant male cotton-top tamarins, *Saguinus oedipus*, to mate's pregnancy. *Horm Behav* 45:84–92
- Ziegler TE, Prudom SL, Zahed SR, Parlow AF, Wegner F (2009) Prolactin's mediative role in male parenting in parentally experienced marmosets (*Callithrix jacchus*). *Horm Behav* 56(4):436–443
- Ziegler TE, Peterson LJ, Sosa ME, Barnard AM (2011) Differential endocrine responses to infant odors in common marmoset (*Callithrix jacchus*) fathers. *Horm Behav* 59:265–270