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Intensity of aggressive interactions modulates testosterone in male marmosets

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Abstract

Androgen is associated with the expression of male-typical behavior, including aggressive behavior, but high levels of androgen may be incompatible with other behavioral systems, such as paternal care. In a variety of species of birds that display paternal care, testosterone (T) levels in males are maintained at low levels, and these levels rise only in response to direct agonistic challenges. This idea has not been thoroughly studied in mammals with biparental care, and we exposed male marmosets (*Callithrix kuhlii*), a monogamous and biparental primate to aggressive interactions with unfamiliar intruders. Urinary levels of T and cortisol (CORT) were monitored prior to and following these interactions. Baseline T was not correlated with variation in aggression in either residents or intruders, and CORT was not affected by the encounters. However, males responded to an encounter with male intruders with changes in T that correlated with the level of aggression displayed by the resident male during the trial. Encounters with male intruders that elicited high frequencies of aggressive displays by the male resident were associated with increased T 2–6 h and 24 h following the encounter, and encounters that had few aggressive displays resulted in no change or a decrease in T concentrations. Intruders did not demonstrate a significant relationship between T and aggression. Thus, the magnitude of the hormonal response is dependent on the intensity of aggression during a male–male encounter, suggesting that elevated androgens are likely to be a consequence, rather than a cause, of aggressive interactions in marmosets.

Keywords: Marmoset; Callitrichid; Testosterone; Aggression; Intruder

1. Introduction

Testosterone (T) affects the development and function of reproductive characteristics in males, including gametogenesis, sexual behavior, aggressive behavior, and maturation of secondary sexual characteristics [1–3]. These features all contribute to male reproductive success. At the same time, however, maintaining high concentrations of T may be associated with considerable costs for males. High concentrations of T are associated with decreased immune function, decreased energy reserves, and decreased paternal investment [4] (cf. Refs. [5,6]). In species whose social systems are characterized by a high variance in male reproductive success, the costs associated with elevated T may be outweighed by the potential that T-mediated traits lead to greater success in siring offspring. In polygynous avian species with little or no paternal care, T titers are maintained at high levels throughout the breeding season to facilitate aggressive interactions for territory and mate acquisition [4,7–9]. However, in monogamous species that show paternal care, androgen levels are high only during the period of territory and mate acquisition, and are low throughout the rest of the year. In spite of the low levels, however, males retain the ability to respond to territorial or aggressive challenges from other males with transient elevations in T, presumably to facilitate aggression during these intruder challenges [4,7–9].

Studies of aggression and testosterone in primates have focused on species that form large troops containing

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multiple males and females. Male aggression in these species is often a result of intraspecific intrasexual conflict. These conflicts are often due to changes in and attempts to maintain hierarchical status. Studies of these species have found that males express very high levels of testosterone and high levels of aggression towards other males during an encounter [10–13]. Although basal levels of T may be low in these species due to overall stable social systems, a conflict results in drastic changes in T concentrations. In fact, the concentrations of T are thought to be linked to the outcome of the interaction, with the dominant "winner" showing increases in T, while the subordinate "loser" shows drastic decreases in T following the interaction [10–12]. While previous studies of primates have focused upon species that have multiple males with varying levels of paternal care, to date no study has examined the hormonal response of primate males to an aggressive encounter in a species that shows high levels of male investment in the offspring.

We tested whether T concentrations in male black tufted-ear marmosets (*Callithrix kuhlii*) predicted aggressive behavior in encounters with unfamiliar males, and whether T levels were altered as a consequence of engaging in an aggressive interaction with a stranger. Marmosets are socially monogamous primates, and have high levels of paternal care [14–19]. The infants are fully dependent on caregivers for 5–6 weeks after birth, and males begin carrying infants at 1 week of age, and are the primary carriers from 3 weeks of age until weaning [20]. Nunes et al. [20,21] have demonstrated that T levels are lowest in males during the period of maximal offspring care, that T is higher in males with less paternal experience, and that T levels are higher in males that carry infants at low rates. Previous behavioral studies have revealed that callitrichids are differentially aggressive towards intruders [22–24]. Specifically, male black tufted-ear marmosets responded with the highest frequency of aggressive displays when exposed to an unfamiliar intruder, they displayed less aggression towards a familiar male, and even less to a female intruder [24]. Male–male aggression in callitrichids appears to differ from other primates previously studied [10–13], because callitrichid conflict appears to be driven by territorial maintenance rather than intragroup conflict to maintain hierarchical status. Male hormonal responses to male–male aggression during territorial encounters in marmosets have not been studied. Although we may predict from other primate studies that an aggressive encounter would be associated with an increase in T, it is unknown whether the hormonal response will be dependent on the sex of the intruder, or on the status of the encounter (i.e.: a win versus a loss).

We used an intruder paradigm [22–24] to test the behavioral and endocrine responses of males to aggressive encounters with conspecifics. Residents were exposed to encounters with adult and juvenile intruders of both sexes, and the intensity of agonistic interactions was monitored throughout the encounter. We expected the intensity of agonistic encounters to be greatest in male–male encounters. If the intruder elicits an aggressive response from the resident (i.e.: poses a territorial challenge to the resident), then exposure to a male intruder should be associated with increases in the resident male's T titers. Since the reaction to the agonistic encounter may be different for an intruder animal than for the resident, we also monitored the intruder's behavioral and endocrine responses to these trials. Finally, because agonistic encounters serve as potent stressors in a wide variety of species [10,12,25,26], we monitored changes in cortisol (CORT) across the phases of the experimental encounters.

2. Methods

2.1. Subjects

2.1.1. Residents

Five pairs of Wied's black tufted-ear marmosets (*C. kuhlii*), consisting of an adult breeding male and his mate, served as resident subjects for the study. The animals were housed at the University of Nebraska at Omaha Callitrichid Research Facility. The animals were maintained in pairs and small family groups in cages measuring at least 1.2×0.9×2.4 m. The cages contained natural branches, a nest tube, a feeding platform, and enrichment devices. Visual access between groups was limited, but olfactory and auditory contact was available with two to three other family groups. For further details of animal husbandry and housing, see Ref. [27].

2.1.2. Intruders

Twenty marmosets were used as intruder subjects in our observations. Thirteen marmosets (six males and seven females) were adult intruders (>24 months) and were breeding adults in their own social groups. Seven intruders (four males and three females) served as juvenile intruders. Juveniles were between the ages of 6 months and 2 years of age, and resided in a social group that contained older adult parents.

2.2. Procedure

2.2.1. Intruder trials

The residents were exposed and habituated to the intruder cage before any testing began by placing the empty intruder cage in each home cage for three 8-h time periods prior to the first test. We avoided testing groups with females in the third trimester of pregnancy or with nursing infants. The resident pair and intruder were first observed during a 10-min pre-trial period. The intruders were removed from their home cage by coaxing them into a small transport cage attached to their home cage. From the transport cage the animals were transferred to an intruder

cage (60×40×40 cm), constructed of wire mesh. The intruder cage eliminated the possibility of physical contact between residents and intruders, while still providing good visual contact between interacting marmosets. After the intruders were transferred to the intruder cage they were allowed to acclimate to the cage for 5 min in an empty room. After this period the intruder was placed in the home cage of the resident pair to be tested.

Two observers were required for the intruder trial, one to record the behavior of the intruder and the resident of the same sex, and one to record the behavior of the other resident. Inter-observer reliability was at least 90% for all trials, as was determined by comparing affiliative behavior of the pair during the trial. Observations occurred at 20-s intervals for 30 min, and were recorded using Observer 3.0® on a laptop computer. Recorded patterns of agonistic and aggressive behavior toward the intruder, affiliative behavior within a pair, and general activity of both the resident and intruder were recorded during each observation as described in Table 1 [24,28]. Following the intruder trial, the intruder was released back into its home cage and the behavior of both residents and intruder was monitored for an additional 10 min. All intruder encounter trials started between 0800 and 0900 h.

Each resident pair was presented with two different adult males, two adult females, and one female and one male juvenile, and 3 weeks separated all trials with an animal, whether the animal served as a resident or an intruder. Additionally, at least 2 days separated all trials in the colony in order to prevent a general disturbance to the colony. Exposure of residents to a male and female intruder was alternated. Control trials were randomly scheduled among experimental trials while leaving 3 weeks between each use of an animal, these trials served as control for both behavioral responses to novel situations, as well as a control for the daily circadian rhythms of hormones to be assayed.

2.2.2. Control resident

An empty intruder cage was placed in the resident cage and observations were performed in the same manner as described above. After the 30-min trial period the intruder cage was removed from the room and a 10-min post observation was conducted.

2.2.3. Control intruder

The intruder was placed in the intruder cage and then placed in a novel empty cage, the size of standard family housing. This trial was used to control for non-encounter related handling effects. Observations were made as described for the experimental trial. The animal was then released into its home cage and observed.

Table 1
Definitions of behaviors collected during the intruder trials for both the residents and intruders (as defined in French et al., 1995; Schaffner and French, 1997)

Behavior	Method	Description
State of resident		
Moving	Instantaneous	The focal animal is traveling to a new position
Rest	Instantaneous	The focal animal is not traveling
Feeding	Instantaneous	The focal animal is consuming food or water
Grooming	Instantaneous	The focal animal is scratching its own body
Playing	Instantaneous	The focal animal is in apparent play behavior alone, or with others, usually includes chase behavior
Social interactions		
Allogrooming	Instantaneous	Grooming or being groomed by the mate
Contact	Instantaneous	The resident pair is in physical contact
Near	Instantaneous	The focal animal is within 10 cm of its mate
Copulating	All occurrences	The focal animal is engaged in mating behavior
Proximity to cage		
Close	Instantaneous	Within 10 cm of the intruder cage
Agonistic responses		
Erh–erh	All occurrences	Vocalization which is a low gutteral sound typically accompanied by attack behavior
Long call	All occurrences	Vocalization which is used as a contact call, long in duration and high in pitch
Twitter	All occurrences	Multi-syllable vocalization that follows a long call
Scent marking	All occurrences	A genital rub on branches or other surfaces
Genital display	All occurrences	Exposing genital area by lifting the tail
Attack	All occurrences	The focal animal charges the cage and bites the wire
Piloerection	All occurrences	The focal animal's hair is extended from body
Intruder demeanor		
Neutral–attentive	Instantaneous	The intruder follows movement but makes no response
Neutral–nonattentive	Instantaneous	The intruder is non-responsive to the actions of the resident
Submissive	Instantaneous	The intruder is cowering and vocalizing in low tones
Agonistic	Instantaneous	The intruder actively attacks and chases the resident

2.2.4. Urine collection

Prior to testing (0600–0800 h), urine was collected from all resident and intruder subjects using the procedures previously outlined in Ref. [29]. Urine was collected every 2 h following the trial from each of the animals tested until 1700 and then again the following morning using positive reinforcement techniques. All samples were centrifuged at 7000 rpm for 2 min to remove debris and the supernatant was transferred to a clean minivial. The samples were then stored at −20 °C until the assays were performed.

2.3. Hormone analysis

2.3.1. Testosterone

T concentrations were measured in samples using an enzyme immunoassay adapted for *C. kuhlii* outlined in Refs. [21,30]. Urine samples (10 μl) were diluted in 1000 μl phosphate-buffered saline (PBS) and incubated for 18 h at 37 °C after the addition of 25 μl β-glucuronidase (Type H2, Sigma, 2700 Fishman units). Samples were then extracted with 5 ml anhydrous diethyl ether, evaporated to dryness, and reconstituted with 2 ml PBS. Extraction efficiency was monitored by the external recovery of ^{3}H-T added to extraction tubes and was 99.9% (n=7). The microtitre plates (Nunc-Immuno Plate MaxiSorp F96) were coated with rabbit anti-T: BSA (#5/98) diluted 1:15,000 in bicarbonate coating buffer. Standards were diluted with PBS and ranged from 1000 to 1.95 pg (ICN Diagnostics). The steroid conjugate (horseradish peroxidase) T-HRP (#11/98) was diluted 1:15,000 in PBS; recovery of the standards was 104.7±10.4%. Intra-assay coefficients of variation for the high and low control pools were 4.33% and 4.16%, respectively (n=9). The inter-assay coefficients of variation for the high and low control pools were 13.84% and 12.57%, respectively (n=9).

2.3.2. Cortisol

CORT concentrations were measured in all urine samples using an enzyme immunoassay developed and validated for use in *C. kuhlii*, as previously described in Refs. [31,32]. Urine samples were diluted 1:6400 with deionized water. Microtitre plates were coated with 50 μl per well of antibody (R4866, raised against a steroid bovine albumin (BSA) in rabbit, diluted to 1:16,000 in EIA phosphate buffer). Standards were diluted in water and ranged from 1000 to 1.95 pg (Sigma). The steroid conjugate (horseradish peroxidase) was diluted in EIA phosphate buffer to a dilution of 1:30,000. Recovery of all standards (range 1.95–1000 pg) added to quality control pools was 101±2%. The intra-assay coefficients of variation for medium and low concentration pools were 4.46% and 3.47%, respectively (n=20). The inter-assay coefficients of variation for the medium and low concentration pools were 14.29% and 17.75%, respectively (n=20).

2.3.3. Creatinine

All hormone concentrations were corrected for the creatinine concentration of each sample to control for fluid intake and output. Creatinine concentrations were measured using a Jaffé end-point assay [33] previously described and validated for *C. kuhlii* [29].

2.4. Data analyses

2.4.1. Behavioral analysis

Since residents experienced two separate intruder encounters with adult intruders, the replicate trials with adult intruders were collapsed into a composite score for each intruder sex. All behavioral data were standardized to a frequency per 10 min to allow direct comparisons between pre-/trial-/post-values. Several aggressive displays and behavioral patterns were summed to produce one composite data point for analysis; this high-level aggression category included the behaviors attack, chase, erh–erh, cage mark, and piloerection [24,28]. A four-factor analysis of variance was used to determine differences among trial conditions, and LSD post hoc tests used to evaluate all between-subjects main effects, and further paired t-tests were used to analyze all interactions. Specifically, for resident behaviors the design was a 2 (intruder sex)×2 (intruder age)×3 (time of the observation: pretrial, during, post trial). A comparison was not made comparing resident's behavioral response to intruder conditions versus the control condition because no aggressive behaviors were displayed during any of the trials in the presence of the empty cage. Intruder demeanors and high-level aggressive behavior by the intruder were analyzed using a 2 (intruder sex)×2 (intruder age)×2 (trial condition: trial, control)×3 (time of the observation: pretrial, during, post trial) design.

2.4.2. Hormone analysis

The hormonal data for both residents and intruders were analyzed using the percent change relative to the baseline morning samples, for the samples collected 2–6 h after the trial (samples averaged together) and samples 24 h after the trial, accounting for both short-term and long-term excretions. These times were chosen based on previous evidence that excretion of CORT and T may commence as early as 2–6 h following a change in the plasma concentrations [30–32]. Changes in T and CORT concentrations of male residents were analyzed using an analysis of variance, specifically 5 (exposure conditions: adult male intruder, juvenile male intruder, adult female intruder, juvenile female intruder, control)×2 (time after the trial: 2–6 h, 24 h). The change in T and CORT for intruder males was tested with a 2 (age of the intruder: adult, juvenile)×2 (condition: intruder trial, control)×2 (time after the trial: 2–6 h, 24 h). The controls were included in the ANOVA in order to determine whether changes in hormone concentrations throughout the day after an intruder trial were

distinct from changes that are associated with normal circadian rhythms [31].

Paired *t*-tests or post-hoc LSD tests were used to explore significant main effects and interaction effects revealed by the ANOVA's. One male resident provided a urine sample that contained concentrations of T that were 15-fold higher than his average hormonal concentrations (25,000 ng/mg Cr, average=1607 ng/mg Cr). This sample was eliminated and only concentrations from the second replicate trial were used in the analysis for this male.

Unbalanced repeated measures SAS Proc Mixed was used to compare the relationships between aggression and the measured hormones, as well as examining the relationship between changes in T and CORT. For these analyses the samples were not collapsed or averaged between trials. Each resident was analyzed using every trial in which they participated, i.e.: two adult males, and two adult females. Hormone samples were averaged for the 2–6 h time period. Analyses first examined the relationship between male resident aggression and T or CORT for all intruders; further analysis restricted the comparison to exposure to intruder males only. Similar tests were used to compare changes in T and CORT over time, as well as relationships between aggression, T, and CORT for intruders. An α of 0.05 was set for all analyses.

3. Results

3.1. Resident responses

3.1.1. Behavioral measures

The occurrence of high-level aggressive behaviors varied as a function of the interaction of the sex of the resident and the sex of the intruder ($F(1,8)=10.5$, $P=0.01$, Fig. 1). Males reacted with a higher frequency of high-level aggressive behavior during exposure to male intruders as compared to female intruders (male intruder: 3.71 ± 0.77 displays/10 min; female intruder: 0.75 ± 0.38 displays/10 min; $P=0.02$). Although residents showed lower rates of agonistic behavior toward juvenile intruders relative to adult intruders, the differences were not significant ($F(1,8)=3.4$, n.s.). Male residents exhibited significantly higher rates of genital displays during the trial and post-trial observations than during the pre-trial observations ($F(2,8)=9.1$, $P=0.02$, pre-trial: 0.09 ± 0.01 displays/10 min; trial and post-trial: 1.16 ± 0.68 displays/10 min). The number of mating bouts significantly increased during and following the trials, compared to the pretrial ($F(2,8)=17.49$, $P<0.001$, pre-trial: 0.04 ± 0.02 bouts/10 min; trial and post-trial: 0.34 ± 0.32 bouts/10 min). No significant differences across trial phases were found for vocalizations or scent marking behavior.

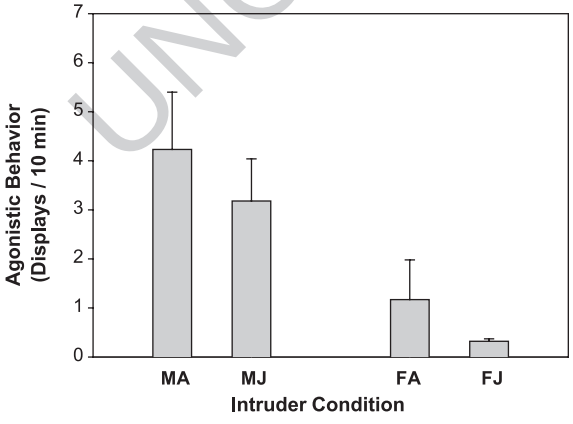

Fig. 1. The number of high-level agonistic behaviors (including: attack, chase, erh–erh, cage mark, and piloerection) displayed by male residents during the intruder trials (intruder conditions: MA=male adult intruder, MJ=male juvenile intruder, FA=female adult intruder, FJ=female juvenile intruder). (Bars are mean±S.E.M., $P<0.05$).

3.1.2. Testosterone

Male residents showed highly variable absolute concentrations of T, and the differences in mean T as a function of intruder condition (adult male, juvenile male, female, juvenile female, or control) were not significant ($F(1,4)=2.5$, n.s.). Additionally, relative concentrations of T, expressed as a proportion of pre-trial concentrations, did not differ due to exposure to different intruder conditions ($F(1,4)=5.6$, n.s.). However, the time after the intruder encounter did affect the proportionate change in T concentration relative to pre-trial levels. Specifically, the change in T from pre-trial was lower 2–6 h after the trial than 24 h after the trial ($F(1,4)=8.38$, $P=0.04$, 2–6 h: $-5.85\pm3.67\%$; 24 h: $8.57\pm3.78\%$). This difference presumably reflects the circadian rhythm of T [30].

While exposure to intruders was not associated with consistent changes in average absolute or relative T concentrations, the intensity of the aggressive interaction in a given encounter with male intruders may serve as a predictor of change in T subsequent to the encounter. Resident males did not show a significant relationship between high-level aggression and T prior to the trial or changes in T 2–6 h or 24 h after the encounter when both male and female intruders were included (prior: $F(1,4)=0.69$, n.s.; 2–6 h: $F(1,4)=1.49$, n.s.; 24 h: $F(1,4)=2.65$, n.s.). When the responses of male residents to male intruders were analyzed several significant relationships were found. High-level aggression of a male resident towards a male intruder was significantly related to T 2–6 h and 24 h following the encounter (2–6 h: $F(1,4)=7.57$, $P=0.05$; 24 h: $F(1,4)=9.02$, $P=0.04$). However, the aggression by the resident was not related to T concentrations of the resident prior to the trial ($F(1,4)=1.68$, n.s.). Fig. 2 indicates that residents tended to show reduced T after trials involving fewer (0–10) high-level aggression displays between residents and male intruders, but large increases in T 24 h after trials involving higher rates of high-level aggression and displays (11–40) (i.e.: attack, chase, erh–erh, piloerection, and cage marking).

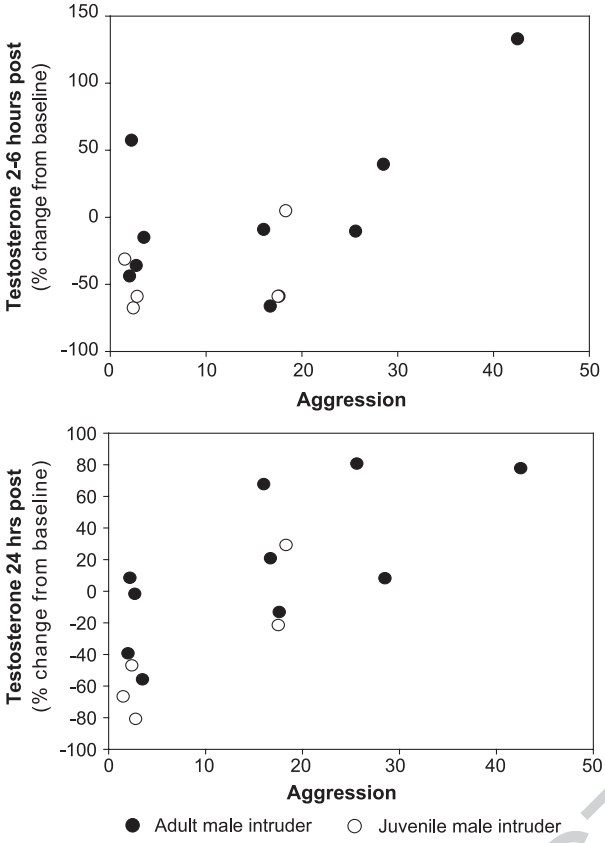

Fig. 2. The relationship between the change in testosterone concentrations 2–6 h (top panel) and 24 h (bottom panel) after exposure to male adult and juvenile intruders and number of high-level agonistic behaviors displayed during the trial by the resident (n=15, P<0.05).

3.1.3. Cortisol

Male residents did not have a differential change in CORT concentrations after exposure to intruders (F(1,4)=2.6, n.s.). However, residents showed a significant change in the concentration of CORT due to the time after the trial (F(1,4)=7.8, P=0.04). The change in concentration 2–6 h after the trial was significantly higher than the change in concentrations 24 h after the trial (2–6 h: 34.34±11.5%; 24 h: 16.77±6.2%, Fig. 3). The control values represent the daily circadian rhythm of CORT, and did not differ significantly from the trial values.

Additionally there were no significant relationships between aggression and CORT prior to, 2–6 h or 24 h after the encounter with male and female intruders (prior: F(1,4)=0.56, n.s.; 2–6 h: F(1,4)=0.81, NS; 24 h: F(1,4)=0.82, n.s.). There were also no relationships between CORT and T when intruders of both sexes were included (CORT 2–6/CORT 24: F(1,4)=2.78, n.s.; CORT 2–6/T 2–6: F(1,4)=0.26, n.s.; CORT 2–6/T 24: F(1,4)=0.53, n.s.; CORT 24/T 2–6: F(1,4)=0.79, n.s.; CORT 24/T 24: F(1,4)=0.90, n.s.; T 2–6/T 24: F(1,4)=2.49, n.s.).

When the responses of residents to male intruders was analyzed we found that aggression displayed by the resident was not related to resident CORT prior to, 2–6 h or 24 h following the encounter (prior: F(1,4)=0.07, n.s.; 2–6 h: F(1,4)=2.67, n.s.; 24 h: F(1,4)=1.81, n.s.). There were also no significant relationships between the changes in hormone concentrations (CORT 2–6/CORT 24: F(1,4)=0.64, n.s.; CORT 2–6/T 2–6: F(1,4)=0.5, n.s.; CORT 2–6/T 24: F(1,4)=1.33, n.s.; CORT 24/T 2–6: F(1,4)=0.55, n.s.; CORT 24/T 24: F(1,4)=0.8, n.s.; T 2–6/T 24: F(1,4)=3.01, n.s.).

We found no significant relationships between the aggression displayed by the intruder and the aggression displayed by the resident (F(1,4)=0.5, n.s.). We also found no significant relationships between the changes in residents' hormones and the aggression displayed by the intruder during the trial (resident T 2–6: F(1,4)=1.2, n.s.; resident T 24: F(1,4)=0.5, n.s.; resident CORT 2–6: F(1,4)=0.87, n.s.; resident CORT 24: F(1,4)=0.32, n.s.). Finally, we found no significant relationships between the intruder's pretrial hormonal levels and the aggression displayed by the resident during the trial (intruder T pre: F(1,4)=0.08, n.s.; intruder CORT pre: F(1,4)=0.42, n.s.).

3.2. Intruder responses

3.2.1. Behavioral measures

Male and female intruders were equally aggressive during trials (F(2,12)=2.17, n.s.), and aggression was not different among adult and juvenile intruders (F(2,12)=1.32, n.s.). As expected, during encounters with residents intruders showed high rates of genital displays (F(2,12)=4.2, P=0.04, trial: 0.09±0.01 displays/10 min; pre- and post-trial: 0 displays/10 min) and twitters (F(2,12)=4.93, P=0.05, trial: 0.3±0.2 twitters/10 min; pre- and post-trial: 0.13±0.08 twitters/10 min). The intruders scent marked significantly more during the post-trial observations than during the pre-trial or trial observations (F(2,12)=5.37, P=0.02, post-trial: 6.69±2.75 scent marks/10 min; pre-trial and trial: 1.22±0.65 scent marks/10 min). Adult intruders also scent marked significantly more

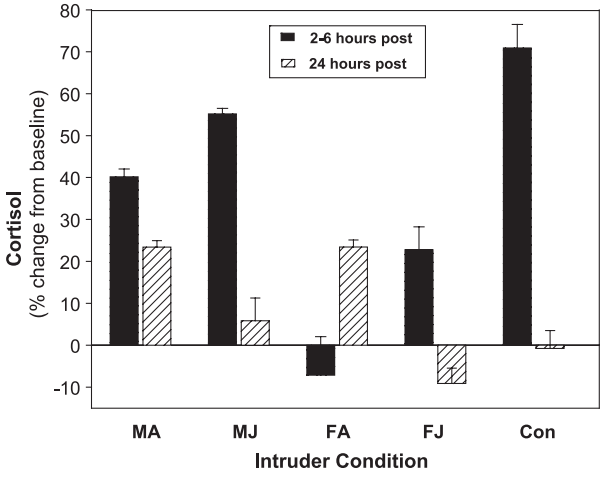

Fig. 3. The percent change from baseline of cortisol concentrations for male residents after intruder trials; there was a significant difference between cortisol 2–6 h post and 24 h after the intruder trial for all conditions, including the control (P<0.05).

than did juvenile intruders ($F(1,7)=8.37$, $P=0.02$, adult: 5.19±2.18 scent marks/10 min; juvenile: 0.9±0.5 scent marks/10 min).

3.2.2. Hormone response

Like male residents, mean levels of T and proportional changes in T following the intruder encounter did not differ for male intruders ($F(1,7)=4.5$, n.s.). Male marmosets also do not appear to have differential stress responses due to use as an intruder. The change in CORT concentrations did not differ due to the time after the trial, or the type of trial ($F(1,7)=2.6$, n.s.).

An examination of T prior to the trial and changes in T 2–6 and 24 h following an intruder trial revealed no relationships with aggression displayed by the intruder during the trial (prior: $F(1,6)=0.43$, n.s.; 2–6 h: $F(1,6)=0.13$, n.s.; 24 h: $F(1,6)=0.01$, n.s.) (Fig. 4). There was also no significant relationship between aggression displayed during the trial and CORT 2–6 and 24 h following the trial (2–6 h: $F(1,6)=0.68$, n.s.; 24 h: $F(1,6)=0.09$, n.s.). However, CORT prior to the trial was significantly related to the amount of high level aggression displayed by the intruder during the trial ($F(1,6)=8.97$, $P=0.04$). While changes in T 2–6 h post and 24 h post were related ($F(1,6)=6.26$, $P=0.05$), there were no other significant relationships between hormone concentrations (CORT 2–6/CORT 24: $F(1,6)=0.68$, n.s.; CORT 2–6/T 2–6: $F(1,6)=0.08$, n.s.; CORT 2–6/T 24: $F(1,6)=1.42$, n.s.; CORT 24/T 2–6: $F(1,6)=0.59$, n.s.; CORT 24/T 24: $F(1,6)=1.2$, n.s.).

We found no significant relationships between the intruder's change in hormonal levels and the aggression displayed by the resident towards the intruder (intruder T 2–6: $F(1,6)=1.1$, n.s.; intruder T 24: $F(1,6)=0.56$, n.s.; intruder CORT 2–6: $F(1,6)=0.12$, n.s.; intruder CORT 24:$F(1,6)=0.32$, n.s.). There were no significant relationships between aggression displayed by the intruder and pretrial levels of residents' hormones (resident T pre: $F(1,6)=0.08$, n.s.; resident CORT pre: $F(1,6)=0.49$, n.s.).

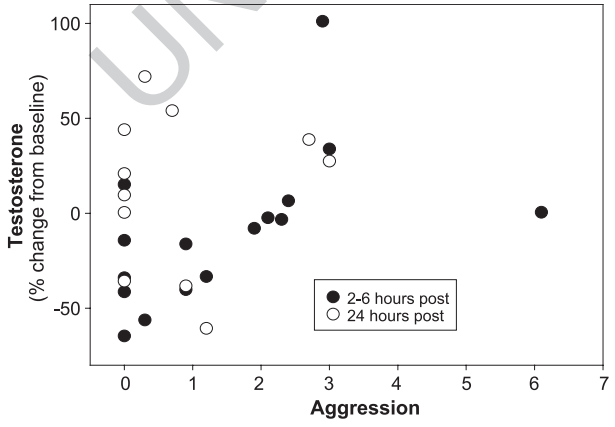

Fig. 4. The relationship between the change in testosterone concentrations in intruder males 2–6 h ($n=15$), and 24 h after an intruder trial ($n=15$), and high-level agonistic behaviors displayed by male intruders (n.s.).

4. Discussion

Black tufted-ear marmoset males respond hormonally to intruder challenges with increases in T *if* the encounter is characterized by high levels of aggressive behavior. Both immediately following the trial (2–6 h) and 24 h after exposure to a male intruder, increases in T were positively related to high levels of aggression, whereas a reduction or no change in T was noted if the resident failed to respond with aggressive displays. Variation in baseline concentrations of T in male residents was not associated with variation in the amount of aggression displayed during the encounter. This suggests that while T is responsive to aggressive encounters, differences in baseline T do not predict aggressive tendencies, at least in the context of a resident male interacting with a male intruder. Therefore, black tufted-ear marmosets exhibit physiological changes in androgen concentrations that vary significantly as a function of the resident's own behavioral response to an intruder. In contrast to T, levels of CORT in male residents were not significantly altered as a consequence of the aggressive interactions.

The change in T is related to the intensity of the aggressive displays by the resident male, rather than the presence of high agonism during the encounter. There is no direct relationship between the intensity of the aggression of the intruder and the change in the resident's T. Additionally, we found no direct relationship between the change in T for the intruder and the intensity of the resident's aggressive displays. Therefore, it appears that the performance of aggression, rather than the presence of or receipt of aggression during an encounter, is associated with changes in T.

The hormonal response of intruders during resident–intruder encounters is very different from the response of the resident. Intruders responded with few *significant* hormonal changes following the encounter, although T did show positive trends associated with aggression 2–6 h following a trial. While residents showed an immediate (2–6 h) and long lasting (24 h) change in T after the trial. Additionally, the number of aggressive displays was substantially reduced in intruders when compared to the frequency of aggression displayed by residents (intruder: 0–6 displays/10 min; resident: 0–40 displays/10 min). Therefore increases in T in association with the amount of aggression performed during the encounter differed based on the status of the animal during the encounter, as seen with other mammals [10–12,25,34,35].

It appears that the significant correlation between aggression performed and changes in post-trial T concentrations is produced by two phenomena: (1) elevated T in residents that behaved aggressively toward intruders, and (2) reduced T in residents that did not behave aggressively toward intruders. The first process suggests that engaging in a competitive, agonistic interaction with a conspecific male elevates androgen titers, perhaps facilitating the expression

of agonistic behavior in encounters in the near future. The mechanisms and functions underlying the second process are less clear. It has been demonstrated that engaging in affiliative interactions does have an impact on endocrine function, at least in the (HPA) axis [36,37]. We do not necessarily suggest that a nonaggressive encounter with an unfamiliar male constitutes an affiliative social interaction in male marmosets, but it could be that T levels are reduced in the aftermath of an interaction that is decidedly non-threatening to the resident. The decreases in T following a non-aggressive encounter may simply reflect the daily circadian rhythm of T [30], or perhaps the male displaying low levels of aggression may be the "loser" of the encounter, as seen in other primates [10–12]. In any event, this second process deserves further attention.

The challenge hypothesis was proposed by Wingfield et al. [4,38] to explain and predict changes in T due to aggressive interactions as a function of the mating systems of the birds. Specifically, in avian systems monogamous males are predicted to show low basal levels of T during times of paternal care, unless directly challenged, in which case the T concentrations increase following the encounter. However, polygynous males exhibit high levels of T throughout the breeding season, and show no changes in T following an encounter because concentrations are already at a maximum. Although our data appears to support the hypothesis that monogamous biparental mammals will show increases in T due to an aggressive challenge, further testing is needed. In order to explore whether T facilitates aggression it is necessary to examine the responses of the residents to future intruder encounters.

In all taxa, agonistic interactions among males vary greatly in the intensity and duration of the interaction. A host of factors contribute to this variation, including behavioral and physiological predispositions of the participants. Tests of mammalian species have not specifically examined the relationship between the amount of aggression displayed by a resident and the testosterone concentrations. Instead mammalian studies have focused on changes in testosterone concentrations following either the presence or absence of an encounter [39,40]. In many ways, the paradigm we used with marmosets is a more dynamic one than that typically tested in the avian or mammalian systems. Marmosets were not screened prior to the tests for aggressive tendencies; therefore both aggressive and non-aggressive intruders were used in our encounters, which produced considerable variability in the intensity of aggression during encounters. Additionally, marmosets have been shown in the past to have low levels of aggression during encounters as compared to other Callitrichids [24]. This variability complicates the nature of the interactions, but presumably our staged intruder encounters more closely resemble variation in the intensity of actual encounters in natural systems.

The interaction of conspecifics during an encounter is often described as a stressful one [25,34]. Many studies have shown a direct increase in the concentrations of CORT following an aggressive interaction such as one modeled by our intruder trial [25,26,34] and these changes in CORT are often associated with changes in T. However, an encounter with intruders in marmosets does not appear to constitute a significant psychosocial stressor, as indexed by HPA function. The changes in CORT after an aggressive encounter do not differ significantly from changes we observed during the control conditions. The similarity of changes in CORT excretion in control trials and intruder trials suggests that we recorded differences attributable to the circadian rhythm [31,32]. These results are similar to those reported in the monogamous titi monkeys, who also display little or no change in CORT following an encounter with an unfamiliar conspecific [41]. Therefore, unlike polygynous primates with low levels of paternal care, such as the olive baboon [10] and squirrel monkey [41], it appears that monogamous primates with a higher prevalence of paternal care like the titi monkey and marmosets, do not respond to intruder challenges with increases in concentrations of stress hormones. Additionally, our data reveal that there is no direct relationship between CORT and T for either the resident or intruder males. Therefore, it is unlikely that differences in T concentrations following the intruder encounter are associated with the suppression of gonadal activity brought about by HPA activity.

In natural populations of marmosets, neighboring social groups interact at the boundary of their territory on a regular basis [18,19,42]. Extra-pair copulations have been noted during some of the encounters, suggesting the possibility of extra-pair fertilization in marmosets. Thus, the critical preconditions favoring the elaboration of intermale aggression and its mediation or facilitation by increases in T are clearly present in marmosets. At the same time, however, elevated T may have a deleterious impact on male fitness. We have demonstrated that the period of maximal paternal effort by males, as indexed by infant carrying scores, is associated with dramatic decreases in T concentrations [21]. Further, males that engage in high levels of paternal care have significantly lower T, relative to males that engage in low paternal effort [20]. The data we present in this paper suggest that regulation of T titers in male marmosets is exquisitely designed to reflect the competing demands of intrasexual aggression and paternal care. Male marmosets do in fact respond to agonistic challenges with increases in T, but the magnitude of the response is conditional upon the intensity of the encounter.

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