

## Post-partum variation in the expression of paternal care is unrelated to urinary steroid metabolites in marmoset fathers

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### ABSTRACT

The organization and activation of maternal care are known to be highly regulated by hormones and there is growing evidence that expression of paternal care is also related to endocrine substrates. We examined the relationship between paternal behavior and steroid hormones in marmoset fathers (*Callithrix geoffroyi*) and evaluated whether hormone–paternal behavior relationships were altered by previous offspring-care experience in males. Based on previous findings, we predicted that testosterone, estradiol, and cortisol would decrease following the birth of offspring and would be lowest during the period of maximal infant carrying. Furthermore, we predicted that post-partum changes in carrying effort and hormone levels would be influenced by the level of offspring-care experience. Carrying effort and other paternal care behaviors underwent temporal changes over the post-partum period, but these patterns were not related to variation in hormone concentrations over the same period. There was a limited effect of offspring-care experience on hormone concentrations, but experience was found to play a role in the expression of paternal care, with experienced fathers engaging in significantly more infant allogrooming than inexperienced fathers. Furthermore, inexperienced fathers increased the frequency of food sharing in response to infant begging across the post-partum period, while experienced fathers displayed consistently low levels. We posit that a combination of experiential factors and an increased role for alloparents in offspring-care led to these changes. However, it appears that hormonal changes may not influence paternal responsiveness in white-faced marmoset fathers and that hormone–paternal behavior relationships are not critically dependent on a male's previous offspring-care experience.

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Parental investment is energetically demanding, and has evolved in species where the benefits of enhancing offspring growth and survival considerably outweigh the immediate costs to the caregiver and the likelihood of future reproductive efforts of the caregiver (Trivers, 1974). Paternal behavior in mammals is uncommon because mammals are defined by the provisioning of milk by the female during lactation (Numan and Insel, 2003); yet, there are a number of mammalian species that display biparental care. Mammalian paternal care is generally seen only in pair-bonded monogamous species (Numan and Insel, 2003), where both parents are required to provide infant care because of high-energy demands on the lactating female. Thus, males may display direct infant care behavior as a way to reduce the energetic burden on the female (Fernandez-Duque et al., 2008).

The organization and activation of maternal care in rodents (Bridges, 1984; Krasnegor and Bridges, 1990) and primates (Fleming et al., 1987; Pryce et al., 1988, 1993) are known to be highly regulated by hormones, most notably the critical role of estradiol, cortisol, and progesterone. There is also growing evidence that expression of paternal care is

related to endocrine substrates (Ziegler, 2000). Several hormones are related to the expression of paternal care in monogamous mammalian species that display biparental care. Peptides, such as oxytocin (Feldman et al., 2010), prolactin (Dixon and George, 1982; Mota et al., 2006), and vasopressin (Wang et al., 1994) are elevated in the circulation when the caregiver displays high levels of contact with offspring. Therefore, an examination of the endocrine factors that are associated with the onset of paternal care can provide insight into common underlying neuroendocrine mechanisms among taxonomic groups, and allow us to determine if there is a common evolutionary pathway underlying the expression of parental care.

Steroid hormones have also been consistently implicated in paternal behavior in avian species. A decline in courtship behavior, and an increase in parental care, is associated with a concomitant decline in androgens in most species that display paternal care (Ketterson and Nolan, 1992; Wingfield et al., 1990). This decline in androgens following the birth of offspring may increase paternal responsiveness and inhibit infanticide (Brown et al., 1995). Testosterone (T) has been implicated in the trade-off between parenting and mating effort in species that show high levels of paternal care (Kuzawa et al., 2009).

There is substantial evidence that levels of T decrease following the birth of offspring in high-investing mammalian fathers. This inverse

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association is seen in several rodent species, including Mongolian gerbils (*Meriones unguiculatus*) (Brown et al., 1995; Clark and Galef, 1999) and Dwarf hamsters (*Phodopus campbelli*) (Reburn and Wynne-Edwards, 1999). However this effect is not universal among rodents, as other species have failed to show this effect (Luis et al., 2009, 2010). Trainor and Marler (2002) found that via aromatization of T, estradiol ( $E_2$ ) stimulates the occurrence of paternal behavior in the California mouse (*Peromyscus californicus*). This may explain how several rodent species display paternal care when T levels are high, and suggests that T does not always suppress paternal behavior, and may actually promote paternal behavior in rodents (Lonstein et al., 2002).

In addition to sex steroid hormones, glucocorticoid hormones have also been implicated in the transition between mating and parenting. Not only is cortisol (CORT) associated with the mother–infant bond (Fleming et al., 1997a, 1997b), but CORT is also related to the expression of male parental care. Male dwarf hamsters (Reburn and Wynne-Edwards, 1999) and expectant human fathers (Storey et al., 2000) experience an increase in CORT concentration prior to birth of offspring. Male black-tufted marmosets (*Callithrix kuhlii*) that carried infants at higher rates had significantly lower CORT in the weeks just after birth, which suggests an inverse relationship. These results support the notion that there are associations between sex steroids, glucocorticoid, and parental care in mammalian fathers.

In monogamous New World monkey (NWM) species that form pair bonds, such as marmosets (Nunes et al., 2000), owl monkeys (Fernandez-Duque et al., 2008), titi monkeys (Schradin et al., 2003), and tamarins (Ziegler et al., 1996), both males and females display high levels of parental care. Infant carrying is a particularly important behavior in marmosets because the young depend on the caregivers for warmth, protection, transportation, and nutrition. The male marmoset displays high levels of infant care, which reduces the burden of the female marmoset. This is important because not only are litters typically composed of rapidly growing twin offspring, but the female marmoset also experiences a highly fertile post-partum ovulation within two weeks after the litter is born (French et al., 1996, 2008; Mustoe et al., 2012). Therefore, the male marmoset's extensive contribution to infant care is essential in the development and survival of the offspring and may facilitate the female's ability to produce future offspring (Fite et al., 2005; French et al., 2008).

Temporal variation in steroid hormones is associated with the timing and expression of paternal care. Lower levels of T were seen in males more involved in postnatal infant care in black tufted-ear marmosets (Nunes et al., 2000) and siamings (*Symphalangus syndactylus*) (Rafacz et al., 2012). A significant decrease in T was also seen during the period of maximal offspring-care in male marmosets, suggesting that steroid hormones and paternal care exhibit temporal changes during the post-partum period (Nunes et al., 2000, 2001). Furthermore, male marmosets that displayed high rates of infant carrying had significantly lower levels of T,  $E_2$ , and CORT than males that display low rates of infant carrying (Nunes et al., 2001). In common marmosets (*C. jacchus*) exposure to infant scent leads to a reduction in circulating T, which suggests that olfactory cues from infants may also play a causal role in the downregulation of T (Prudom et al., 2008). However, a reduction in T in marmoset fathers occurs even in the absence of continued post-partum exposure to infants (Nunes et al., 2000). Thus, the temporal pattern of hormone concentrations in marmosets appears to co-vary with changes in paternal responsiveness.

In prior work it was suggested that a consequence of changes in hormone levels, as a result of offspring-care experience, may be that male marmosets devote more energy to infant care. This increased investment in paternal care is a corollary with a decrease in mating effort outside of the well-established pair bond (Nunes et al., 2001). Male black tufted-ear marmosets that had raised four or more litters had significantly lower levels of T at the time the male engaged in maximal levels of paternal care than males that had raised only one or no litters, even when controlling for age-related differences in T

(Nunes et al., 2001). Furthermore, CORT levels in male cotton-top tamarins without offspring-care experience were significantly greater than CORT levels in experienced males (Ziegler et al., 1996); yet in inexperienced male common marmosets, CORT was reduced as compared to males with offspring-care experience (Mota et al., 2006). These results suggest that the role of T and CORT changes with additional offspring-care experience.

The purpose of the investigation was to examine the relationships between paternal behavior and steroid hormones in male white-faced marmosets (*C. geoffroyi*), and to evaluate the influence of offspring-care experience on these factors. The current study was designed to evaluate whether changes in testosterone, estradiol, and cortisol are associated with the transition to behavioral fatherhood in male marmosets. The white-faced marmoset is a socially monogamous New World monkey species endemic to Brazil. The breeding male provides extensive infant care, with infant care responsibilities shared between the male and female of the breeding pair, and older siblings. Females experience post-partum estrus  $17.45 \pm 2.22$  days after the litter is born (Mustoe et al., 2012), and the modal litter size is two offspring.

To the extent that urinary steroid metabolites are associated with the expression of paternal care in other mammalian species, we predicted that (1) T,  $E_2$ , and CORT would decrease following birth of offspring, and (2) would be significantly lower in males that displayed high levels of carrying effort. Furthermore, it was predicted that (3) gonadal and adrenal steroid hormones would decrease during the period of maximal infant carrying, and (4) that post-partum changes in carrying effort and hormones levels would be influenced by the level of offspring-care experience.

## Method

### Subjects

Offspring-care behavior exhibited by six adult male marmosets was evaluated during each male's first and second paternal litter (total of 12 litters), corresponding to inexperienced and experienced fathers respectively. Details of family group composition can be found in Table 1. Breeding of white-faced marmosets was regulated by the American Zoological Association (AZA), as detailed in the Species Survival Plan (SSP). Marmosets were housed in family groups at the Callitrichid Research Center (CRC) at the University of Nebraska at Omaha (UNO). Animals were kept in large indoor wire-mesh enclosures ( $1.0 \times 2.5 \times 2.0$  or  $2.0 \times 2.5 \times 2.5$  m), equipped with a sleeping hammock, natural branches for climbing and various enrichment material. Visual access was restricted between enclosures, but auditory and olfactory cues were not. Colony rooms at the CRC were maintained on a 12 h:12 h light:dark cycle and at a temperature range between 19 °C and 22 °C. For all dietary and husbandry protocols please refer to Schaffner et al. (1995). The University of Nebraska at Omaha/University of Nebraska Medical Center Institutional Animal Care and Use Committee evaluated and approved all procedures: Protocol #: 07-033-05-FC.

### Behavioral observations

Data on infant care behavior were analyzed for the first eight post-partum weeks. Twenty minute focal animal observations were conducted at a randomly selected time between 1000 and 1600 h by a trained observer. All observers were trained to achieve a level of proficiency ( $\kappa > 0.95$ ) on scoring infant care behavior. Focal surveys were conducted  $2.5 \pm 0.8$  (SD) times per week using Observer 8.0 software. Animals were exposed to humans several times throughout the day, and were given several minutes to habituate to the presence of humans prior to an observation.

Infant-carrying behavior was defined as the infant clinging to the body or pelage of the parents or alloparents (i.e. older offspring in

**Table 1**  
Family composition.

Male	Litter #	Litter	
		Offspring	Alloparents
Acorn	1	2.0	0
Acorn	2	1.1	2
Antonio	1	2.0	0
Antonio	2	2.0	1
Manny	1	1.1	0
Manny	2	0.2	2
Raymond	1	1.1	0
Raymond	2	1.0	2
Rosco	1	0.2	0
Rosco	2	1.1	2
Spanky	1	0.2	0
Spanky	2	1.1	2

Note. Details of family group composition. Only the first two litters for each male were evaluated. The number of males and females within each litter (# of males is noted before the period and # of females is noted after), and the number of alloparents present at the time of birth are listed.

the family group) and was measured in minutes per hour of observation. Although infant-carrying behavior is the most visible and energetically costly form of paternal investment in offspring-care, several other paternal behaviors were also analyzed. These included infant-allogrooming, anogenital licking (AGL), attempted infant transfers, infant removals, infant retrievals (combined attempted and successful), food sharing in response to infant begging, steals (combined attempted and retrievals), and infant transfers. Details of paternal behaviors can be found in Table 2. An examination of this multifaceted behavior is essential if we are to ascertain the overall level of paternal effort exerted during infant care.

#### Urine collection

First-void urine samples were collected from the adult male marmosets, between 0700 and 0900 h an average of three times per week, beginning two weeks before and continuing eight weeks after the birth of the litter, using non-invasive techniques described by French et al. (1996). Subjects were trained to urinate into small pans for food rewards. Urine samples were centrifuged at 2000 rpm for 5 min to separate sediment from the sample. The supernatant was transferred to a clean vial and stored at  $-20^{\circ}\text{C}$  pending assay.

#### Hormone assays

Testosterone, estradiol, and cortisol concentrations were measured by conducting thirteen assays for each hormone. Displacement

curves of halving dilutions for a *C. geoffroyi* male quality control urine pool and hormone standards were parallel in the 10–90% binding range, such that a difference in dilution resulted in an equivalent difference in the calculated concentration. Urine samples were diluted appropriately to fall in this range.

Testosterone and estradiol levels, from hydrolyzed and extracted samples (for details, see Fite and French, 2000; Nunes et al., 2000), were measured by enzyme immunoassay (EIA). Ten  $\mu\text{l}$  of the urine sample was diluted in 1000  $\mu\text{l}$  of phosphate buffered saline (PBS), which then incubated for 12 h at  $37^{\circ}\text{C}$  after the addition of 20  $\mu\text{l}$  of  $\beta$ -glucuronidase (Sigma #0876). Free and unconjugated steroids were extracted in 5 ml of diethyl ether. The ether was evaporated under a gentle air stream and reconstituted in 1 ml of PBS. Extraction efficiency was measured by assessing the recovery of  $^3\text{H-T}$  and  $^3\text{H-E}_2$ . Recovery estimates were 90.0% for T and ranged from 40.9 to 56.5% for  $\text{E}_2$ . Hormone levels were adjusted accordingly to account for procedural losses of steroid.

To quantify urinary T, microtiter plates were coated with T antibody (Ab) (5/98), diluted 1:5000 in bicarbonate coating buffer, and incubated for 12 h. Testosterone standards were diluted in PBS ranging from 1000 to 7.8 pg/well. Labeled T conjugate (horseradish peroxidase; HRP, 12/03), was diluted 1:140,000 in PBS. After the 12 hour incubation, 50  $\mu\text{l}$  of PBS was added to each well, followed by 50  $\mu\text{l}$  of the extracted urine samples or T standards. After 50  $\mu\text{l}$  of HRP was added, the plates were set to incubate for 2 h. We then separated free and bound hormones and added an EIA substrate (ABTS,  $\text{H}_2\text{O}_2$ ). Absorbance at 405 nm was measured in a microplate reader. Intra-assay coefficients of variation (CV) for high and low concentration pools were 4.34% and 3.75%, respectively. Inter-assay CVs for the same high and low concentration pools were 14.92% and 17.90%, respectively.

For the  $\text{E}_2$  assay, microtiter plates were coated with  $\text{E}_2$  Ab (K4972), diluted 1:15,000 in bicarbonate coating buffer, and incubated for 12 h.  $\text{E}_2$  standards were diluted in PBS ranging from 2500 to 19.5 pg/well. Labeled  $\text{E}_2$ -HRP (12/03) was diluted 1:160,000 in PBS. The  $\text{E}_2$  EIA procedure was identical to the T EIA procedure. Intra-assay CVs for high and low concentration pools were 5.75% and 3.48%, respectively. Inter-assay CVs for the same high and low concentration pools were 13.28% and 15.92%, respectively.

For the CORT assay, microtiter plates were coated with CORT Ab (3.6.07), diluted to 1:25,000 in bicarbonate coating buffer, and incubated for 12 h. CORT standards were diluted in PBS ranging from 1000 to 7.8 pg/well. Labeled CORT-HRP (R4866) was diluted 1:30,000 in PBS. The CORT EIA procedure was identical to the T and  $\text{E}_2$  EIA procedure. Intra-assay CVs for high and low concentration pools were 6.13% and 3.91%, respectively. Inter-assay CVs for the same high and low concentration pools were 9.64% and 11.40%, respectively.

We expressed the mass of T,  $\text{E}_2$ , and CORT in  $\mu\text{g}$  per mg of creatinine (Cr) to control for variation in the solute concentration of the urine

**Table 2**  
Paternal care ethogram.

Activity	Operational definition
Allogroom	An individual licks an infant's body or manipulates an infant's pelage by parting its fur with its hands and removing particles with hands or mouth.
Anogenital licking (AGL)	An individual licks an infant's anogenital area.
Attempted transfer	One individual attempts to transfer (or take) an infant from the individual currently carrying that infant, but fails to complete the transfer. Failure is not due to the current carrier's resistance.
Carrying	Infant clings to the body or pelage of a parent or alloparent.
Removal	The individual carrying an infant attempts to remove the infant onto cage wire or onto any other object/substrate other than another monkey. Combined both attempted and successful removals.
Retrieval	One individual attempts to retrieve an infant from cage wire or any other substrate (other than another monkey), and is not able to pick up the infant (or the infant does not climb on), or is successful. Combined attempted and successful retrievals.
Food shares	An individual allows a begging infant to share a solid food item.
Steal	One individual attempts to transfer (or take) an infant from the current carrier, against the will of the current carrier, and the carrier either successfully resists the transfer or does not resist the transfer. Combined attempted and successful steals.
Transfer	One individual transfers (or takes) an infant from the individual currently carrying that infant. There is no resistance from the carrier losing the infant.

Note. Paternal care ethogram detailing the objective classification of each paternal behavior examined.

samples. Creatinine was measured using a standard Jaffé reaction colorimetric assay (French et al., 1996).

### Statistical analysis

A weighted paternal effort index (PEI) was calculated by summing the total time carrying one or both infants per observation. This value was then converted into infant-carrying minutes per hour. The value is weighted because some litters contained only one infant. Thus, potential maximum values of the PEI for litters of two were twice as large as maximum values for a litter of one, reflecting the greater energetic effort (and potentially paternal motivation) associated with carrying two vs. one infant. These values were then averaged within two-week time intervals.

We compared PEI, other paternal care patterns, and hormone concentrations in inexperienced males and males with offspring-care experience. Offspring-care behavior and hormone concentrations from six males were analyzed from each male's first two litters. The model comparison approach was used to evaluate the effect of offspring-care experience on paternal care and hormone concentrations over the post-partum period. Contrast coded predictors (Factor 1: experienced vs. inexperienced; Factor 2: first eight post-partum weeks) were used to create an analysis of variance (ANOVA) model. Several mixed-model ANOVAs were used to evaluate the effect of offspring-care experience on urinary steroid metabolite concentrations and several features of paternal care.

## Results

### Post-partum variation in paternal care and hormone concentrations

Marmoset males carried infants at significantly different rates over the post-partum period [ $F(3, 30) = 5.36, P = 0.004$ ] (refer to Fig. 1A), with the highest rates occurring during post-partum weeks 3–4. PEI scores during post-partum weeks 3–4 were significantly greater than PEI scores during post-partum weeks 1–2 and 7–8 and marginally greater than PEI scores during post-partum weeks 5–6. PEI scores were significantly greater during post-partum weeks 5–6 than post-partum weeks 7–8 and 1–2.

Temporal changes in hormone concentrations were not related to the overall expression of paternal care. Testosterone levels [ $F(3, 27) = 2.21, P > 0.05$ ] (refer to Fig. 1B), estradiol levels [ $F(3, 27) = 0.38, P > 0.05$ ] (refer to Fig. 1C), and cortisol levels [ $F(3, 27) = 0.88, P > 0.05$ ] (refer to Fig. 1D) in males did not change significantly over the post-partum period. However, the temporal pattern in T excretion does resemble the inverse of the temporal variation in carrying effort.

Marmoset males tended to display varying rates of anogenital licking (AGL) across the post-partum period [ $F(3, 36) = 2.73, P = 0.06$ ] (refer to Fig. 2A). Although the omnibus test was not significant, trend analysis indicated that there was a significant quadratic trend [ $F(1, 12) = 4.89, P = 0.05$ ]. AGL during post-partum weeks 1–2 occurred significantly less frequently than AGL during post-partum weeks 3–4, and moderately less frequently than AGL during post-partum weeks 5–6.

The frequency with which fathers engaged in food sharing in response to infant begging increased across the post-partum period [ $F(3, 30) = 3.41, P = 0.03$ ] (refer to Fig. 2B). Food sharing during post-partum weeks 7–8 occurred significantly more frequently than food sharing during post-partum weeks 1–2, 3–4, and 5–6. Marmoset males also engaged in varying rates of retrievals over the post-partum period [ $F(3, 30) = 4.42, P = 0.01$ ] (refer to Fig. 2C). Retrievals during post-partum weeks 1–2 occurred significantly less frequently than retrievals during post-partum weeks 5–6 and 7–8. Retrievals during post-partum weeks 3–4 also occurred significantly less frequently than retrievals during post-partum weeks 5–6 and 7–8.

Marmoset males also displayed varying rates of removals across the post-partum [ $F(3, 30) = 2.92, P = 0.05$ ] (refer to Fig. 2D). Removals during post-partum weeks 7–8 occurred moderately more frequently than removals during post-partum weeks 1–2 and 3–4. Removals during post-partum weeks 5–6 occurred moderately more frequently than removals during post-partum weeks 1–2.

The frequency of attempted infant transfers is shown in Fig. 2E. Fathers decreased the amount of attempted transfers across the post-partum period [ $F(3, 30) = 4.75, P = 0.01$ ]. Attempted transfers during post-partum weeks 1–2 occurred significantly more frequently than attempted transfers during post-partum weeks 5–6 and 7–8, and moderately less frequently than attempted transfers during post-partum weeks 3–4. Marmoset males did not display varying rates of allogrooming [ $F(3, 30) = 0.81, P > 0.05$ ] (refer to Fig. 2F), infant transfers

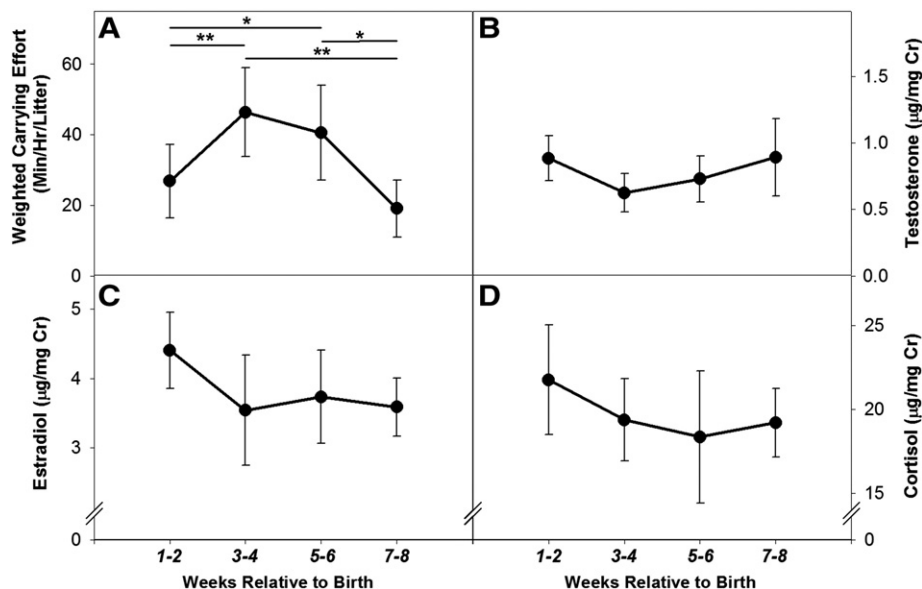
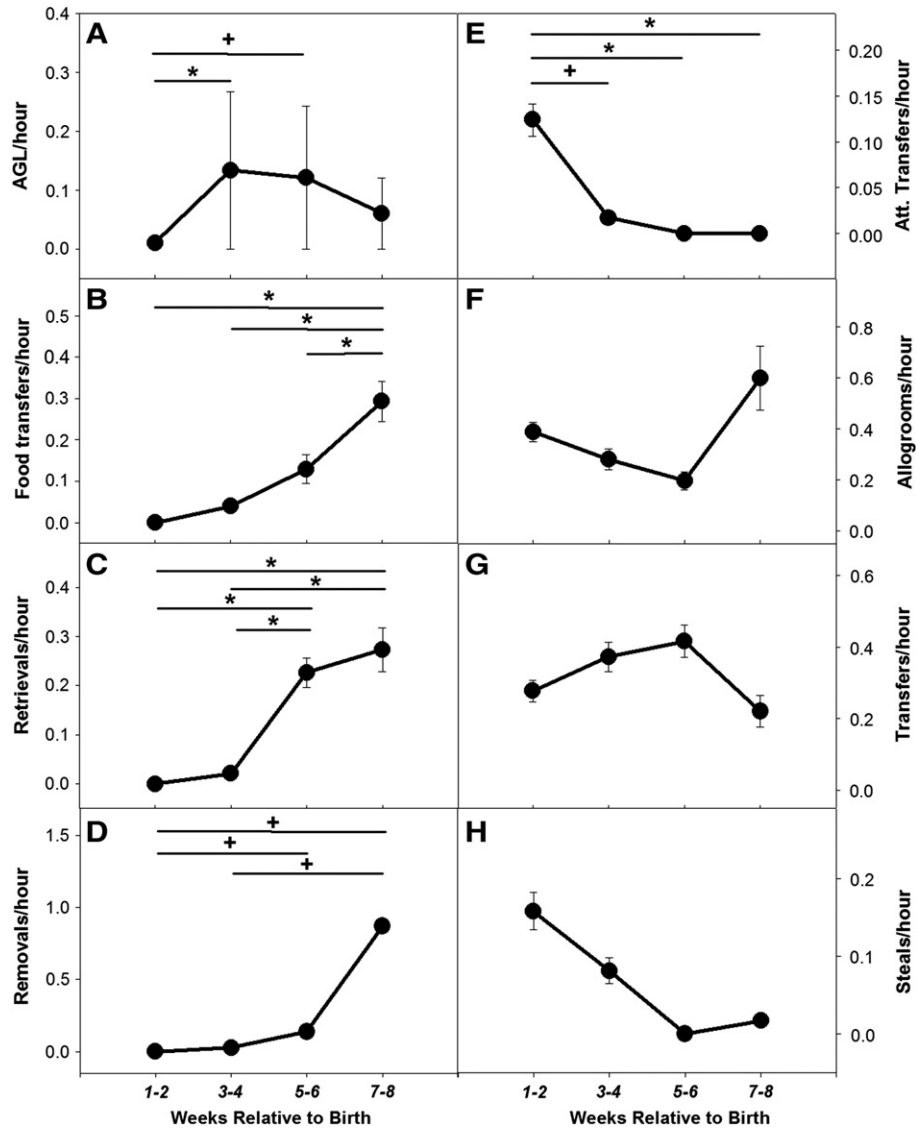


Fig. 1. (A) Infant carrying effort, (B) urinary testosterone concentration, (C) urinary estradiol concentration, and (D) urinary cortisol concentration of male marmosets (*Callithrix geoffroyi*) following the birth of a litter. Asterisks indicate significant differences (\*  $< 0.05$ , \*\*  $< 0.01$ ).



**Fig. 2.** The frequency which male marmosets (*Callithrix geoffroyi*) engaged in (A) anogenital licking (AGL), (B) food sharing in response to infant begging, (C) infants retrievals, (D) infant removals, (E) attempted infant transfers, (F) infant allogrooming, (G) infant transfers, and (H) infant steals during the first two months after birth. A detailed description of each behavior can be found in Table 2. Asterisks indicate significant differences (\* < 0.05, + < 0.1).

[ $F(3, 30) = 0.78, P > 0.05$ ] (refer to Fig. 2G), or infant steals [ $F(3, 30) = 2.32, P > 0.05$ ] (refer to Fig. 2H) over the post-partum period.

*Effect of offspring-care experience on paternal care and hormone concentrations*

Inexperienced male marmosets did not display a distinct pattern of PEI after parturition as compared to males with experience [ $F(3, 36) = 1.66, P > 0.05$ ] (refer to Fig. 5A). However, trend analysis revealed that inexperienced males did display a more robust increase in PEI over the early post-partum period and a less robust decrease in PEI over the late post-partum period, compared to males with offspring-care experience [ $F(1, 12) = 4.71, P = 0.05$ ]. Inexperienced male marmosets tended to display a distinct pattern of T excretion during the post-partum period, compared to males with offspring-care experience [ $F(3, 27) = 2.83, P = 0.06$ ] (refer to Fig. 5B). Male marmosets without offspring-care experience exhibited a quadratic pattern of T excretion over the post-partum period, with the lowest levels of T excretion occurring during post-partum weeks 3–4 and 5–6, which is the period with the highest expression of PEI [ $F(1, 9) = 6.05, P = 0.04$ ]. Experienced males displayed consistently low levels of T excretion over the post-partum period.

Additionally, male marmosets without offspring-care experience did not display a distinct pattern of  $E_2$  [ $F(3, 27) = 1.19, P > 0.05$ ] (refer to Fig. 5C) or CORT [ $F(3, 27) = 1.56, P > 0.05$ ] (refer to Fig. 5D) change over time, as compared to males with offspring-care experience. Inexperienced male marmosets did not have significantly different excreted levels of average T [ $F(1, 5) = 0.24, P > 0.05$ ],  $E_2$  [ $F(1, 5) = 4.91, P > 0.05$ ], or CORT [ $F(1, 5) = 2.22, P > 0.05$ ] across the first eight post-partum weeks than experienced male marmosets.

Marmoset males with experience displayed significantly more infant allogrooming than males without experience [ $F(1, 12) = 6.53, P = 0.03$ ] (refer to Fig. 3). However, there were no significant differences between experienced males and inexperienced males in the frequency of AGL, infant-transfers, attempted infant-transfers, infant-retrievals, infant-removals, food sharing, or infant-steals. However, there were indications that experience modulates the temporal changes in the frequency of food sharing in response to infant begging across the post-partum period. Male marmosets with offspring-care experience displayed a distinct pattern of food sharing as compared to inexperienced males [ $F(3, 36) = 2.80, P = 0.05$ ] (refer to Fig. 4). Inexperienced males displayed significantly more food sharing at post-partum weeks 7–8 than post-partum weeks 1–2, 3–4, and 5–6, while males with

offspring-care experience displayed consistently low levels of food sharing across the post-partum period. The presence of alloparents at the birth of a male's second paternal litter was highly confounded with experience, though, the age of the alloparents ( $274.67 \pm 50.0$  days) at the birth of the second litter varied between families.

## Discussion

In male marmosets, carrying effort underwent temporal changes over the post-partum period, and this pattern remained steady regardless of prior offspring-care experience. Carrying effort increased to peak levels during post-partum weeks 3–4 and 5–6. The post-partum variation in AGL followed the same pattern as carrying effort, such that the highest number of AGL occurred coincident with the highest rates of infant carrying. This is not surprising because marmosets perform AGL during successful infant transfers, which coincide with the onset of a carrying bout. The interpretation of the results that AGL frequency varies over time should be made cautiously because the analysis indicated that there was a trend.

The number of attempted transfers was highest during post-partum weeks 1–2, which suggests that fathers have a high motivation to carry infants, but are unsuccessful in receiving the infants from the current carrier. We know that peak rates of maternal carrying in black-tufted ear marmosets occur during the first two post-partum weeks and peak rates of paternal carrying occur during post-partum weeks 3–4 (Nunes et al., 2001). The decrease in attempted transfers after post-partum weeks 1–2, and subsequent increase in AGL and carrying rates in white-faced male marmosets suggests that fathers have taken on a greater proportion of parental responsibility during post-partum weeks 3–4.

Male marmosets vary their parental tactics in relation to infant needs as the infants mature in post-natal development. As infants are weaned by female marmosets, males increase the amount of infant removals and decrease the amount of time carrying infants. Fathers will also retrieve infants significantly more during the second month post-partum, which is not surprising because infants are almost always clinging to a caregiver during the first month of life. This increase in removals and retrievals may be a mechanism by which fathers promote independence in rapidly-growing infants, but fathers also stand ready to retrieve infants if they are in danger. During the second post-partum month infants become more autonomous and fathers modify their behavior to account for this change. This includes increasing the amount of food sharing due to infant begging. During this time infants are beginning to learn how to forage for food, but still require considerable contributions from caregivers. Thus, temporal variations in paternal behaviors indicate that fathers modify their parental tactics in relation to infants needs.

The post-partum temporal variation in carrying effort was unaffected by offspring-care experience. However there were differences in the

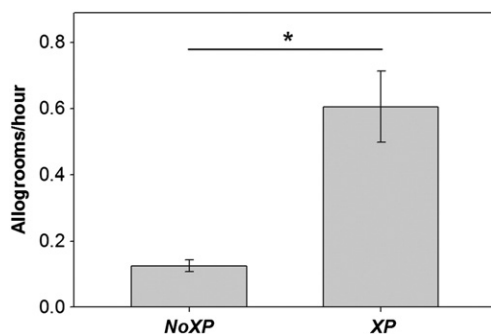


Fig. 3. Comparison of male marmosets (*Callithrix geoffroyi*) following the birth of a litter, contrasting the frequency of infant allogrooming between a male's first (NoXP) and second (XP) paternal litter. Asterisks indicate significant differences (\* < 0.05).

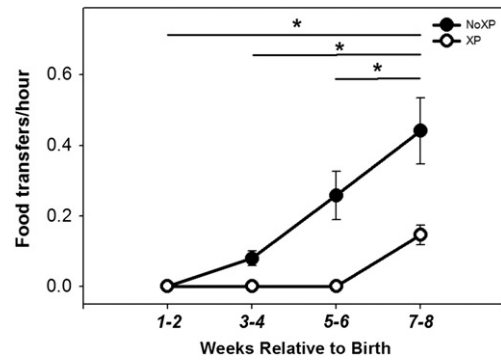


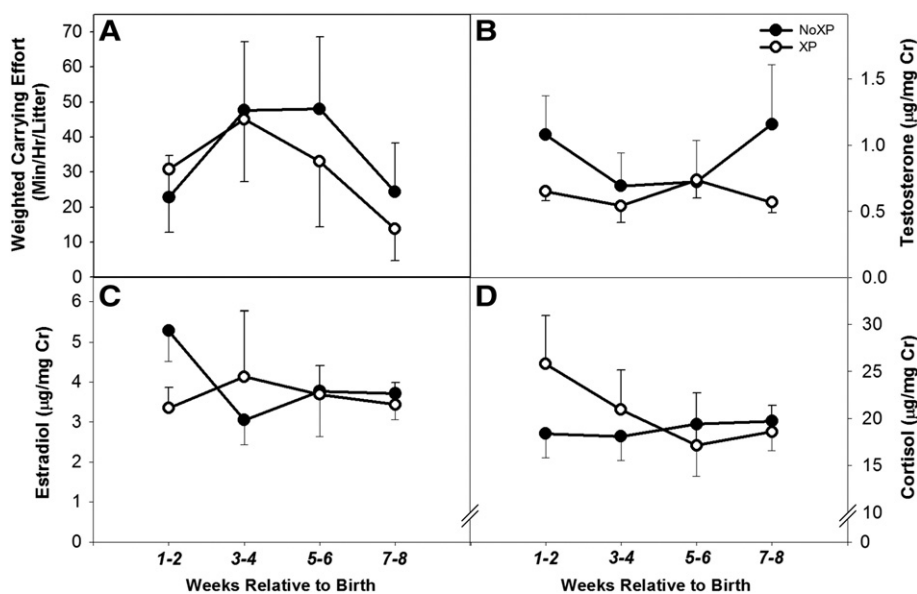
Fig. 4. Comparison of male marmosets (*Callithrix geoffroyi*) following the birth of a litter, contrasting the frequency of food sharing in response to infant begging between a male's first (NoXP) and second (XP) paternal litter. Asterisks indicate significant differences (\* < 0.05).

expression of other paternal behaviors as the result of offspring-care experience. Offspring-care experience was associated with the frequency of infant allogrooms performed by the father, with experienced fathers engaging in nearly four times the amount of allogrooms as inexperienced fathers. This suggests that experienced fathers display an increase in paternal effort in this facet of offspring care due to a reduction in the expression of others areas of caregiving.

The number of times a father shared a food item to a begging infant was also modulated by experience, such that only inexperienced fathers increased the number of times they shared a food item over the post-partum period. This suggests that either experienced fathers share food items with begging infants less because fathers have learned to share food prior to infant begging or that food sharing responsibilities may be shifted to alloparents. In marmosets, older offspring tend to remain with the family group and aid in caregiving, thus reducing the burden on the parents. Nunes et al. (2001) found that alloparents displayed similar levels of carrying effort as the adult female from post-partum weeks three through eight, in black tufted-ear marmosets. Therefore, some of the variation in paternal investment may be accounted for by the involvement of alloparents. In our sample, experience level was confounded with the total number of caregivers in the family group. Thus, we are unable to dissociate whether variation in paternal care is brought about directly by experience-related changes in males or simply by changes in group demography and the number of potential caregivers available to interact with infants. It is likely that a combination of paternal experience and an increased role for in alloparents in caregiving led to the differential pattern of paternal effort in male marmosets.

Excreted hormone levels in male marmosets did not change significantly across the post-partum period. In contrast to findings in black tufted-ear marmosets (Nunes et al., 2001), male white-faced marmosets did not experience a significant post-partum decline in T levels. These results are not consistent with the view that T is negatively related to paternal behavior. It should be noted that the role T plays in mammalian paternal behavior can vary depending on the species in question. Although a decrease in T levels generally results in an increase in paternal care and a decrease in aggressive behaviors, in rodents T has been shown to maintain paternal behavior. Trainor and Marler (2001) have shown that castration in California mice led to a reduction in male parental care, and that this effect may be the result of aromatization of T to E<sub>2</sub> (Trainor and Marler, 2002). Male marmosets exhibit relatively high levels of E<sub>2</sub> under normal conditions (Nunes et al., 2002), and both circulating and excreted E<sub>2</sub> is increased following a GnRH challenge in male marmosets (Nunes et al., 2002). Yet, patterns of E<sub>2</sub> excretion in male white-faced marmosets were not related to the expression of paternal care.

Aside from the Clark and Galef (1999) finding that paternal care is diminished in castrated Mongolian gerbils that received T, there has



**Fig. 5.** Comparison of male marmosets (*Callithrix geoffroyi*) following the birth of a litter, contrasting (A) infant carrying effort, (B) urinary testosterone concentrations, (C) urinary estradiol concentration, and (D) urinary cortisol concentrations between a male's first (NoXP) and second (XP) paternal litter.

been limited support to the claim the testosterone regulates paternal care. However, there is experimental evidence to suggest that male parental care is not regulated by gonadal steroids. Hume and Wynne-Edwards (2005) found that in biparental dwarf hamsters, castration does not reduce paternal responsiveness. Wynne-Edwards and Timonin (2007) suggest that although there may be an inverse relationship between T and expression of paternal care, that there may not be a causal link, and that the hormone–behavior associations may be due to other challenges coincident with the transition to fatherhood.

In addition to the finding that T and E<sub>2</sub> concentrations did not change across the post-partum period in male marmosets, there were also no significant post-partum changes in CORT, which indicates that CORT may not be related to the expression of paternal care in white-faced marmosets. Furthermore, offspring-care experience was not a significant predictor of CORT excretion during the post-partum period. The relationship between CORT levels and parental responsiveness has been shown in female primates, yet the nature of the cortisol–behavior relationship differs depending on the species. Human mothers with high post-partum cortisol were more attracted to, and more accurate at recognizing, their newborn infant's body odor (Fleming et al., 1997b), while female western lowland gorillas (*Gorilla gorilla gorilla*) and female savannah baboons (*Papio hamadryas*) had higher CORT levels during times when they had less contact with infants (Bahr et al., 1998; Bardi et al., 2004). In other cases, it has been suggested that increased glucocorticoids during pregnancy may prepare a mother for infant interactions (Fleming et al., 1997b; Nguyen et al., 2008). An increase in early post-partum CORT may play a preparatory role for paternal care in males as well (Storey et al., 2000). In males, increased CORT levels may function to enhance paternal reactivity and responsiveness. Pup-feeding (Carlson et al., 2006) and infant retrievals (Bales et al., 2006) are strongly associated with an increase in CORT levels, whereas licking and grooming are inversely related to CORT levels. Findings in human fathers indicate that CORT decreases in men that spend more time with their infants (Storey et al., 2011), and is significantly lower in fathers than non-fathers (Berg and Wynne-Edwards, 2001; Gettler et al., 2011b). This is consistent with previous work in black tufted-ear marmosets, which found that low effort males had significantly higher CORT levels during the early post-partum weeks than high effort males (Nunes et al., 2001). However, there is no evidence to suggest that CORT is related to paternal reactivity or offspring-care experience in white-faced marmosets.

Although offspring-care experience was not a significant factor influencing the temporal changes in steroid hormones in white-faced male marmosets following the birth of infants, the data did indicate that T concentrations may follow a temporal pattern inverse to carrying effort only in inexperienced fathers. In male marmosets without offspring-care experience, excreted levels of T were lowest during the period of maximal infant carrying, while in experienced male marmosets T levels remained consistently low across the post-partum period. This suggests that in white-face male marmosets the role of T in the expression of carrying effort may diminish as males gain offspring-care experience. Although the overall trend was that T decreased during the period when paternal effort was at its peak, we must conclude that patterns of carrying effort and other paternal care behaviors are not necessarily related to variation in steroid metabolite excretion over the post-partum period and that these hormone–paternal behavior relationships are not critically dependent on a male's previous offspring-care experience.

The socioendocrinology of paternal care in cooperative breeding non-human primates has several parallels to male parental care in humans. Pairbonded men (Kuzawa et al., 2009) and fathers (Berg and Wynne-Edwards, 2001; Gettler et al., 2011b; Gray et al., 2007; Muller et al., 2009; Storey et al., 2011) show a decrease in bioavailable T as compared to non-fathers and single men. The amount of effort a father devotes to childcare is also related to T levels, with low levels of T seen in fathers who exert the greatest effort (Alvergne et al., 2009); it was suggested that the key factor in the suppression of T was the father-child interaction (Gettler et al., 2011a). Yet, the finding that male marmosets experience a reduction in T levels in the post-partum period regardless of whether infants are present or not (i.e. stillborn infants) during this period (Nunes et al., 2000) suggests that direct exposure to, and interaction with infants is not necessary to produce these endocrine changes in all cases. Still, there has not been a clear resolution to the question regarding the direction of the relationship between T and paternal care: does a decrease in T concentration influence the expression of paternal care or does an increase in the expression of paternal care lead to a decrease in T concentration? Unfortunately, due to the limited scope of the current study we are unable to determine the direction of this relationship. Therefore, a continuing examination of the endocrine factors that regulate paternal care from a variety of taxonomic groups may be able elucidate the underlying neuroendocrine mechanisms that influence offspring-care, and reveal how

endocrine influences on paternal care may have evolved during the development of mating systems in cooperatively-breeding social species.

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