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Hormonal stimulation and paternal experience influence responsiveness to infant distress vocalizations by adult male common marmosets, *Callithrix jacchus*

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ABSTRACT

Parental experience and hormones play a large role in the common marmoset (*Callithrix jacchus*) father's care of their offspring. We tested the effect of exogenous estradiol or testosterone on the responsiveness of common marmosets to respond to infant distress vocalizations and whether males who haven't become fathers yet (paired males) would have increased responsiveness to infant distress calls with either steroid or whether parental experience is the most important component for the onset of paternal care. Sixteen male marmosets (8 fathers, 8 paired males) received a vehicle, low dose or high dose of estradiol and additional 16 males were tested with testosterone at three doses for their response either to a vocal control or a recording of an infant distress call for 10 min. Without steroid stimulation fathers were significantly more likely to respond to the infant distress stimulus than paired males. Low dose estradiol stimulation resulted in a significant increase in fathers' behavioral response towards the infant distress stimulus but not in paired males. Fathers also showed a significant increase in infant responsiveness from the vehicle dose to the estradiol low dose treatment, but not to the estradiol high dose treatment. Testosterone treatment did not show significant differences between infant responsiveness at either dose and between fathers and paired males. We suggest that neither steroid is involved in the onset of paternal care behaviors in the marmoset but that estradiol may be involved in facilitating paternal motivation in experienced fathers.

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1. Introduction

Becoming a parent involves a change in the perception of infants and in the motivation to respond to infant sensory cues. Our basis for understanding the organizational effects involved in the onset of maternal care behaviors has come from years of studying the female rat, *Rattus norvegicus* (Rosenblatt, 1969). Female virgin rats will either avoid unknown pups or attack them (Dulac et al., 2014; Stern, 1983). Female pregnant rats begin to show an increase in responsiveness to pup sensory cues just prior to parturition and continuing from birth through to weaning (Mayer and Rosenblatt, 1984; Bridges, 1975). The cascade of hormonal changes occurring prior to parturition has been shown in the female rat to be involved in the induction of maternal behavior (Moltz et al., 1970; Zarrow et al., 1971).

In female mammals multiple endocrine changes occur during pregnancy and at parturition that are associated with an increase in spontaneous expression of maternal care (Bridges, 2015). Estrogens play an essential role in the induction of maternal care in mammals. This has been demonstrated in many species, including the rat, mice, and ewes (Siegel and Rosenblatt, 1975; Ribeiro et al., 2012; Poindron et al., 1988). Estradiol is also involved in enhancing the acquisition and retention of behavioral preferences for pup isolation calls (Caras, 2013). Additionally, the actions of other hormones such as progesterone, prolactin and oxytocin are dependent upon exposure to estrogens (Bridges, 2015). Little is known about the effect of estradiol on the onset of paternal behavior in males. If estrogen works in males as it does in females, estrogen should increase parenting motivation in fathers and in non-fathers to respond to infant auditory calls. In a bi-parental rodent, the effect of estrogens has been shown to be via testosterone aromatization into estradiol in the brain (Kirkpatrick et al., 1994; Lee and Brown, 2002).

Testosterone, however, has primarily been shown to have a negative relationship with paternal care and is low in bi-parental rodents following the birth of their offspring (Bales and Saltzman, in press). Infant crying is a primary modality of infant communication and a few studies have investigated testosterone reactivity to audiotaped or simulated infant distress. Human males with higher testosterone are less responsive to novel infant cries (Fleming et al., 2002). However, testosterone also has been shown to have a role in the onset of paternal care through its aromatization to estradiol in the bi-parental California mouse (*Peromyscus californicus*) (Trainer and Marler, 2001). In many species, testosterone

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has an inverse relationship with prolactin where prolactin is associated with paternal care (see Ziegler et al., 2009).

Common marmoset fathers have physical and hormonal changes while their mates are pregnant (Ziegler et al., 2009). Males gain weight during the gestation period of their mate and lose weight while performing infant care behaviors and this appears to be under the control of prolactin (Ziegler et al., 2006; Ziegler et al., 2009). Prolactin, estradiol, testosterone and cortisol increase during pregnancy. Testosterone declines significantly following birth of infants and has an inverse relationship with prolactin. Testosterone also significantly declines in fathers after smelling the scent of their dependent infants while estrogens increase (Prudom et al., 2008; Ziegler et al., 2011). In marmosets, and the closely related cotton-top tamarin, *Sauginus oedipus*, males have very high levels of estrogens circulating and excretion into the urine (Ziegler et al., 2000; Ziegler et al., 2009). In the male cotton-top tamarin, estradiol is mainly of gonadal origin and therefore aromatized by testosterone (Ziegler et al., 2000). However, there is no evidence of an induction of paternal behavior in this species without becoming a father.

We have developed a motivation test for our marmosets where males can respond to infant distress calls (Zahed et al., 2008). Fathers who have experienced at least one birth are significantly more responsive to the infant cries than males who have never had their own infants even though they have participated in carrying younger siblings (Zahed et al., 2008; Ziegler et al., 2009). Therefore, either experience or hormonal induction is causing the increase in response to infant distress cries. We gave long acting estradiol or testosterone to fathers or males who had not become fathers yet (paired males) to determine if estradiol or testosterone (through aromatizing to estradiol) would increase male's response to infant distress cries. We hypothesize that if the paired males increase their responsiveness behavior towards infant distress calls when given estradiol or testosterone treatment, then we would expect the steroid to be involved in the onset of paternal behaviors. If not, then it would add support for experience as a father being the most important condition for the onset of paternal care.

Table 1
Marmoset males as fathers (experienced) and paired males (inexperienced) in paternal care with their age, the number of sibling sets they have raised and number of offspring sets raised.

Male ID#	Experience level	Male age (years)	Sibling sets raised	Offspring sets raised
cj0863	Experienced	8.71	4	7
cj0955	Experienced	7.79	2	3
cj1301	Experienced	4.53	2	2
cj1271	Experienced	4.73	2	4
cj1305	Experienced	4.3	0	3
cj1157	Experienced	6.04	1	9
cj1237	Experienced	5.1	3	3
cj1263	Experienced	4.77	1	4
cj1229	Experienced	4.77	0	3
cj1049	Experienced	6.53	2	5
cj1115	Experienced	6.48	1	8
cj1483	Experienced	3.18	0	2
cj1403	Experienced	3.97	2	2
cj1560	Experienced	2.57	1	2
cj1427	Experienced	3.96	1	2
cj1533	Experienced	3.27	0	2
cj1450	Inexperienced	2.41	1	N/A
cj1489	Inexperienced	2.15	0	N/A
cj1479	Inexperienced	2.21	0	N/A
cj1381	Inexperienced	2.97	2	N/A
cj1065	Inexperienced	6.4	9	N/A
cj1285	Inexperienced	4.44	3	N/A
cj1197	Inexperienced	5.24	2	N/A
cj1099	Inexperienced	6.57	2	N/A
cj1163	Inexperienced	5.26	0	N/A
cj1165	Inexperienced	5.26	1	N/A
cj1213	Inexperienced	4.8	3	N/A
cj1345	Inexperienced	4.2	3	N/A
cj1430	Inexperienced	2.83	1	N/A
cj1490	Inexperienced	2.38	0	N/A
cj1053	Inexperienced	6.36	3	N/A
cj1239	Inexperienced	5.29	3	N/A

2. Materials and methods

2.1. Subjects

The study used a total of 32 adult male common marmosets: 16 parentally experienced (Mean ± SD: 5.04 ± 1.68 years) and 16 parentally inexperienced (Mean ± SD: 4.3 ± 1.59 years), Table 1. There was no significant difference by age between the two groups ($t = 1.3$, $df = 30$, $P = 0.21$). All subjects were housed in social groups comprised either of a pair mate or a family (pair mate and their offspring of various ages) in the Marmoset Colony of the Wisconsin National Primate Research Center, University of Wisconsin-Madison. Subjects and their social groups were housed with 12-h light:dark cycles (6:30–18:30 light), a steady temperature of 27 °C, and humidity approximately 40%. The marmosets were fed twice daily at approximately 0800 h and 1300 h in standardized meals consisting of marmoset chow (Mazuri 5MI6, LandO'Lakes) and supplemental fruit, mealworms, and vegetables. Water was provided ad libitum. Cage size varied between 1.22 × 0.61 × 1.83 m (family groups) and 0.6 × 0.91 × 1.83 m (pairs). This study was reviewed and approved by the Graduate School Animal Care and Use Committee at the University of Wisconsin-Madison and the experiments were conducted in accordance with international standards on animal welfare as well as being compliant with national regulations. Adequate measures were taken to minimize pain or discomfort.

2.2. Study design

All study subjects were tested for their behavioral response to a pre-recorded infant distress vocalization and to a pre-recorded artificial control acoustic stimulus (vocal control). The 32 subjects were divided into two categories based on whether or not they had yet experienced the birth of their own offspring. Sixteen of the subject males had previously sired and provided paternal care for their own offspring ("fathers") and were housed with their pair mate and offspring for the duration of this study, and the remaining 16 males had never sired their own offspring ("paired males") and were housed with only their pair mate for the duration of this study. Subjects in both the "fathers" and "paired males" categories had previous alloparenting experience in their natal groups prior to being paired with their own mate before the start of this study, Table 1. (Mean ± SD [range]: $N = 16$ fathers 1.38 ± 1.15 [0–4] sibling litters, $N = 16$ paired males 2.06 ± 2.21 [0–9] sibling litters). Fathers had raised 2 to 9 litters of their own offspring prior to the start of this study (Mean ± SD: $N = 16$ fathers 3.81 ± 2.29 [2–9] offspring litters).

Within the two conditions, fathers ($N = 16$) and paired males ($N = 16$) were each further sub-divided according to hormone treatment (estrogen or testosterone) at three different levels for each subject (vehicle, low dose, high dose), resulting in two groups of eight animals for each hormone treatment, Fig. 1. Each animal was given two days of habituation to the testing cage prior to the start of treatments and each animal received one of the three levels of hormone treatment in randomized order. Three days following hormone treatment, each male was tested for his behavioral response to one of the two auditory stimuli (vocal control, infant distress call) selected at random. He was returned to his social group immediately following behavioral testing, and two-to-four days later, he was tested with the other auditory stimulus. Thus, each animal in each experience condition was tested for behavioral response under five different treatment levels, once for each possible combination of auditory stimulus (vocal control, infant distress call) and hormone level (vehicle, low dose, high dose). Each male was tested at