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Exposure to infant scent lowers serum testosterone in father common marmosets (*Callithrix jacchus*)

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Common marmoset (*Callithrix jacchus*) males are bi-parental non-human primates that show extensive paternal behaviour. Fathers are in direct sensory contact with their infants during the natal period. We found that fathers exposed to isolated scents of their infant displayed a significant drop in serum testosterone levels within 20 min after exposure, whereas parentally naive males did not. These data suggest that infant's scent may have a causal role in regulating paternal testosterone in their fathers. This is the first study to demonstrate that olfactory cues have an acute effect on paternal care.

Keywords: paternal care; testosterone; infant scent; parenting; olfactory

1. INTRODUCTION

While much is known about maternal care, little is known about paternal care (Bridges 2008). Fathers can contribute significantly to the positive outcome of infant and child development (Lamb 1981; Brott 1999). Common marmosets (*Callithrix jacchus*) are cooperative breeding primates, where, similar to humans, the male and the female, as well as any siblings present, participate in infant care. Infants require transportation and genital stimulation for excretion during the first 4–6 weeks. Therefore, males are in close sensory contact with their infants starting from the first day of birth.

Male marmosets have previously been shown to be hormonally responsive to infants. Dixson & George (1982) found that prolactin levels were higher when fathers were carrying offspring prior to blood sampling than in fathers who were not (see also Mota & Sousa 2000). Other members of the family Callitrichidae have shown decreased testosterone with parenting. *Callithrix kuhlii* showed decreased levels of urinary testosterone with increased infant carrying in two-week increments of first morning voids (Nunes *et al.* 2001). Individual males exhibited decreases in urinary testosterone with increased paternal experience. In a related species, father cotton-top tamarins, *Saguinus*

oedipus, males showed decreased testosterone and other androgens during the infant care period. However, testosterone levels increased during their mate's periovulatory period even while fathers were carrying infants (Ziegler *et al.* 2004*a*,*b*). Thus, testosterone levels appear to be responsive to the social environment.

Experienced father marmosets spend considerable time in olfactory contact with their infants from birth to weaning (6–8 weeks), providing ample opportunity for reinforcement of positive paternal behaviour in response to infant cues. Marmosets are highly responsive to olfactory scents, and testosterone levels can be manipulated with odours of ovulatory females (Ziegler *et al.* 2005). This study demonstrated that primates show the same acute rise in testosterone in response to a sexual stimulus, as do other mammals.

Human fathers tend to have lower basal levels of androgens than non-fathers, suggesting that fatherhood results in chronically decreased androgen levels during child rearing (Gray 2006, 2007). However, males did not show an acute decrease in testosterone after 20 min of play, implicating that proximity alone is not sufficient to produce an acute change in testosterone while parenting. When presented with infant stimuli, human males who have experienced couvade (male pregnancy symptoms) also have shown the greatest decrease in testosterone (Storey *et al.* 2000). Fathers with decreased testosterone have a greater need to respond to infant stimuli compared with non-fathers or males who did not have offspring of their own when tested with infant cries (Fleming *et al.* 2002).

Understanding what causes experienced fathers to show an acute and extended decrease in testosterone after spending time with their own offspring may provide insights into positive parental care. We therefore tested the common marmoset to determine whether infant scent alone could affect the hormonal levels of fathers.

2. MATERIAL AND METHODS

Six paternally experienced male marmosets were housed with their mate and offspring at the Wisconsin National Primate Research Center, Madison, WI, USA. Five adult paternally inexperienced males (non-fathers) paired with adult unrelated females were used as controls. Control female pregnancies were prevented via monthly injections of estrumate. The ages of the experienced males ranged from 3 to 9 years while the range was 3–5 years for the inexperienced males. Marmosets were on a 12 L : 12 D cycle with lights on at 06.30 (for details see Saltzman *et al.* 1997). All subjects were housed in $0.6 \times 0.91 \times 1.83$ m cages and equipped with a metal nest-box, wooden dowels and other tactile items. Marmosets are socially housed and are allowed auditory, visual and olfactory contact with other marmosets. Testing met all guidelines set by the Animal Care and Use Committee of the Graduate School at the University of Wisconsin, Madison, WI, USA.

All infant scents were collected as urogenital rubs between 10 and 15 days post-partum. Ground glass stoppers were rubbed lightly over the urogenital area of the infant (Ziegler *et al.* 2005). Three hundred microlitres of ethanol: water degassed solution was washed over the collected scent and pipetted into a microcentrifuge tube for storage at -80°C until testing. Scents for experienced males were collected from their own infants 3–5 days prior to testing and frozen until testing. Scents for inexperienced males were collected from unfamiliar infants using the same methods.

Males were removed from their home cages between 13.00 and 14.00 in their nest-box and placed in a clean nest-box with no visual, olfactory or auditory contact with other marmosets for a 10 min washout period. After 10 min, a wooden disc with 100 µl of either infant scent or vehicle was placed in the nest-box. Stimuli were presented in a crossover design with half the males receiving infant scent first and the other half receiving vehicle first. The males were tested 4 days apart when the infants were three weeks. No males were carrying infant immediately prior to removal from the home cage for testing. The males were allowed to touch, lick,
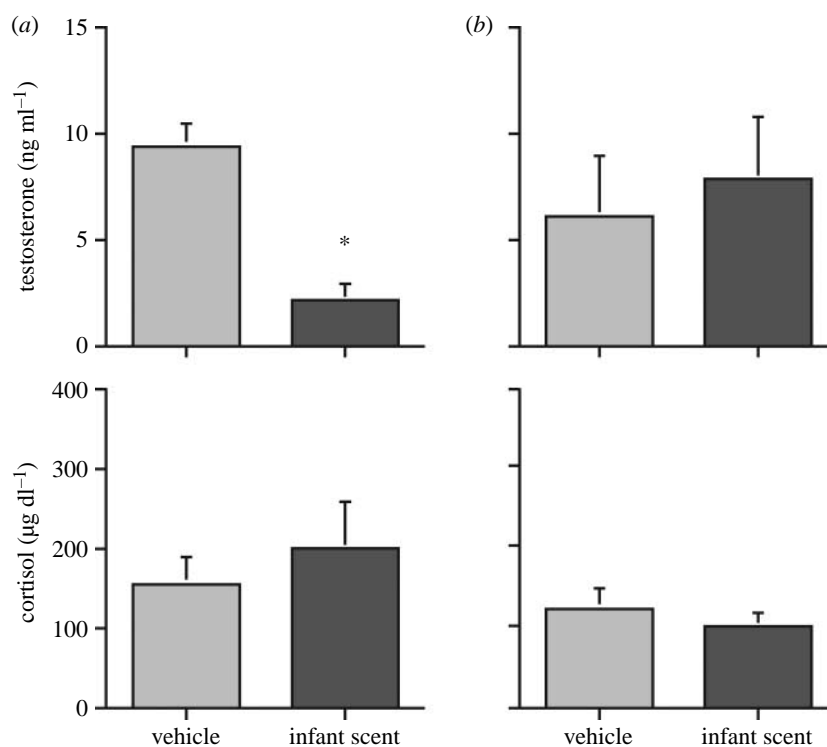
Figure 1. Mean±s.e.m. levels of testosterone and cortisol for paternally (*a*) experienced and (*b*) inexperienced male common marmosets. Hormone levels were measured in serum samples drawn 20 min following the initial presentation of either an infant scent (male's own infant scent for experienced males and a novel infant scent for inexperienced males) or a vehicle control scent. Testosterone showed a significant decrease in levels with exposure to the infant scent for the experienced males; $t = 4.87$, $p = 0.005$. Asterisk indicates a significant difference between infant scent for the experienced fathers, $*p = 0.005$.

smell or scent-mark the disc for 10 min and then the disc was removed, and the males remained in the testing nest-box for an additional 10 min. After 20 min from initial exposure to the infant scent, the males were removed and a 0.3 ml blood sample was taken via femoral puncture within 3 min. The sample was then spun and the serum was separated and frozen until assayed.

Before analyses, all serum samples were processed as 200 µl, extracted with 5 ml diethyl ether and were dried. The steroids were resuspended in 1 ml of methanol for solid phase extraction. The samples were dried and resuspended in 30 µl for HPLC separation. Solid phase extraction and HPLC separation were reported in Ziegler *et al.* (2004*a*,*b*). Cortisol eluted at 4.4 min and testosterone at 13.2 min. Samples were collected as fractions at 3.8–5.5 and 13–15 min, respectively, dried and resuspended to the original volume (200 µl) prior to assay as described below.

Testosterone and cortisol levels were determined using enzyme immunoassays as described in Ziegler *et al.* (2005). For testosterone, 15 µl were assayed for each sample. The samples were assayed in three different assays with an intra-assay coefficient of variation (CV) of 4.83 and an inter-assay CV of 12.22. For cortisol, 10 µl were assayed for each sample and all the samples were run in two assays. Inter- and intra-assay CVs for cortisol in our laboratory are 2.57 and 1.50, respectively.

3. RESULTS

Testosterone was significantly lower in parentally experienced males after exposure to their infant's scent compared with vehicle (paired t-test $= 4.87$, $p = 0.005$). Parentally inexperienced males showed no significant change in testosterone after exposure to a novel infant scent compared with vehicle (paired t-test $= -0.56$, $p = 0.61$; figure 1). Cortisol levels did not change between exposures to infant scent versus vehicle for either group (experienced fathers: $t = -1.32$, $p = 0.24$; inexperienced males: $t = 1.44$, $p = 0.22$; figure 1). There were no differences between the testosterone and the cortisol levels for vehicle exposure for experienced and inexperienced males. Additionally, levels for

the two hormones were in the range of concentrations found for testosterone and cortisol for male common marmosets in a previously reported study (Ziegler *et al.* 2005). These data suggest that infant scents have an acute effect of lowering the testosterone levels in fathers but not in non-fathers.

4. DISCUSSION

Our results demonstrate that odour cues from infants can have an acute effect on lowering their father's testosterone levels compared with a control scent. Although decreases in testosterone were seen previously in human males, no causal effect of infant scent (olfactory) was known to affect the testosterone levels in fathers.

Since testosterone is often thought of as an 'aggression' hormone, chronic decreases may be essential for positive infant care. However, there is a contradiction of decreased testosterone in males while parenting and their need to protect themselves, mates and territory that appear to require increased testosterone. Decreases in testosterone due to castration in bi-parental dwarf hamsters (*Phodopus campbelli*) did not result in parenting changes (Hume & Wynne-Edwards 2005), but did in the California mouse (*Peromyscus californicus* Trainor & Marler 2001). Testosterone influence on parenting behaviours may be through its conversion to oestradiol.

Trainor & Marler (2002) showed that testosterone is aromatized to oestradiol in the brain to facilitate parental behaviours in the California mouse. Oestradiol, a well-known maternal hormone, may facilitate the success of males in this species to provide

exemplary paternal care. This implication may be true for the common marmoset as well. However, testosterone is most likely aromatized into oestradiol in the marmoset father in the gonads as well. The related cotton-top tamarin has peripheral aromatization of testosterone to oestradiol (Ziegler *et al.* 2000). Injections of testosterone in the tamarin result in increase in both testosterone and oestradiol, whereas blocking testosterone synthesis in the gonads lowers oestradiol. In the present study, testosterone was lowered in as little as 20 min after exposure to isolated infant scent, without the presence of the infant. The flexibility of this system allows marmosets and tamarins to be more responsive to acute social needs. Aromatization of testosterone to oestradiol would allow males to still increase their testosterone in times of need, such as defending territory or mates (Ross *et al.* 2004). Aromatization of testosterone to oestradiol has been shown in the brains of male rhesus monkeys (Bonsall *et al.* 1983; Roselli *et al.* 1987), but oestradiol has not been shown to significantly alter reproductive behaviour in males.

Parentally naive males showed no changes in testosterone from a novel infant scent to the vehicle control. This could be due to lack of paternal experience or that only one's own infant can affect the testosterone levels in males. This trigger of one's own infant may explain why with the cooperative nature of the marmoset and exposure to infants throughout childhood, hormonal changes do not occur until the male is a father.

This is one of the few studies to clearly demonstrate a causal role of infant cues in regulating paternal testosterone levels. Since this effect was found with the presentation of scent alone, isolated olfactory cues must work to stimulate a change. Further investigation into the role of infant scent cues and their role in regulating parental hormones is needed.

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