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Hormones and Behavior 47 (2005) 56–64

Hormones and Behavior

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# Neuroendocrine response to female ovulatory odors depends upon social condition in male common marmosets, *Callithrix jacchus*

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Received 23 January 2004; revised 25 July 2004; accepted 11 August 2004
Available online 27 October 2004

**Abstract**

Male mammals show rapid behavioral and hormonal responses to signals from sexually receptive females. However, rapid endocrine responses to female signals have not been observed in a nonhuman primate. Here, we tested the behavioral and hormonal response of male common marmosets (*Callithrix jacchus*) to isolated scent secretions from ovulatory females or to vehicle control scent. Fifteen males were tested in their home cage for behavioral and hormonal responses. These males showed increased investigative and arousal behaviors to the ovulatory scent compared to the vehicle scent. Time sniffing the scent substrate and the duration of erections were significantly elevated in relation to the vehicle scent. Thirty minutes after presentation of ovulatory scent, males showed a significant increase in testosterone compared to the vehicle, but there was no difference in cortisol values. To better control for scent presentation, 15 additional males were tested under a controlled scent exposure. Current social housing condition influenced the male's testosterone response to the ovulatory scent. Single and paired males showed significant increases in testosterone levels with the ovulatory scent but did not increase cortisol levels. Single males also showed the highest change in testosterone with the ovulatory scent, but fathers showed no changes. These results indicate that a rapid hormonal response to sexually arousing cues occurs in marmosets, and the data suggest that a male's social condition influences how he responds to sexually relevant cues.


*Keywords:* Testosterone; Cortisol; Primate; Marmoset; Chemical signal; Social condition

## Introduction

The sexual arousal process, or appetitive response in male mammals, is usually due to stimulation by female sensory cues that activate brain areas such as the medial preoptic nucleus and the paraventricular area of the hypothalamus (Beach, 1956). These areas have been shown to be involved in male sexual behavior (Everitt, 1990, Paredes, 2003). The neuroendocrine system is also activated with rapid increases in pituitary gonadotropins and blood testosterone levels. Thirty to forty minutes after copulation, bulls (Katongole et al., 1971), miniature pigs (Ellendorff et al., 1975), rabbits (Saginor and Horton, 1968), and hamsters (Macrides et al., 1974) all showed significant increases in testosterone levels.

However, the neuroendocrine response is not necessarily related to the act of copulation or consumatory process, but to the sensory cues provided by the female. The most studied sensory cues of sexually receptive females are visual and olfactory. Conflicting results have been reported on testosterone stimulation by showing erotic films to men (for review, see Kruger et al., 1998). For instance, one study indicated increased blood testosterone levels within the first

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doi:10.1016/j.yhbeh.2004.08.009

10 min after presentation of a sexually arousing film (Stoleru et al., 1993), while in another study, testosterone levels remained unchanged to visual sexual stimulation (Carani et al., 1990). Male mice showed an increase of LH within 10 min of having a sexually receptive female placed in the cage (Bronson and Desjardins, 1982). The response occurred whether the male was sexually satiated prior to the introduction of the female or not and whether the male was allowed to copulate or not. In hamsters, the odors of vaginal discharge in the absence of other female cues produced an increase in plasma testosterone within 30 min (Macrides et al., 1974). Odors appear to be important for sexual arousal in nonhuman primates as well. The male lesser mouse lemur responds to urinary chemosignals from estrous females with an increase in testosterone after chronic (2–4 weeks) olfactory stimulation (Perret and Schilling, 1995). Male rhesus monkeys respond to chronic exposure (over several weeks) of sexually receptive females by showing increased testosterone production, whether the females were estrogen-treated ovariectomized females (Elvira et al., 1982; Gordon et al., 1978) or normal cycling females (Rose et al., 1972). All of these studies show long-term testosterone response. A rapid acute testosterone response to sexually attractive odors has not been reported in a nonhuman primate.

The common marmoset, *Callithrix jacchus*, is behaviorally responsive to sexually attractive females and their odors. Male marmosets prefer scent marks from ovulatory females to those of luteal-phase females or noncycling females (Smith and Abbott, 1998). Males respond to female proceptive behaviors and odors with an increase in tongue flicks, mounts, and ejaculation (Kendrick and Dixson, 1983). They also use olfactory cues from females to coordinate their sexual activity with female endocrine status (Dixson, 1998). Hypothalamic lesions of male marmosets in the medial preoptic area (MPOA)/anterior hypothalamus (AH) are associated with diminished sexual behaviors (Lloyd and Dixson, 1988). Indeed, the MPOA is critical in many mammalian species for the regulation of sexual arousal (see Paredes, 2003, for review).

Our previous work using functional magnetic resonance imaging (fMRI) indicates an activation of the MPOA/AH when paired housed males were presented with scent mark odors from novel ovulating females compared to ovariectomized females (Ferris et al., 2001). Many other areas of the brain were stimulated differentially during presentation of ovulatory odors as well (Ferris et al., 2004). However, behavioral and hormonal responses to sexually relevant odors were not tested during these studies. Male marmosets do respond to female receptive scents with sexual behavior under normal housing conditions (Kendrick and Dixson, 1983). Our fMRI study predicts that the marmosets should also show an activation of the pituitary gondotropin–testosterone response to the sexually relevant odors.

Marmosets live in cooperatively breeding groups of 3 to 15 individuals in northeastern Brazil (Stevenson and Rylands, 1988). Typically, only one female reproduces, and the breeding male remains with the family to help raise infants. Offspring remain in their natal group past puberty to assist in caring for younger offspring. Although the family group appears to be critical in rearing offspring, it is also common in this species for males and nonreproductive females to assess the reproductive condition of neighboring groups of marmosets (Lazaro-Perea, 2001). From moderately long-term studies of wild common marmosets, it appears that breeding males do remain with the same breeding female over several pregnancies (Digby, 1995; Lazaro-Perea, 2001). In captivity, males form pair bonds with their mated females and will respond aggressively to female intruders (Harrison and Tardif, 1989), but are less likely to show aggressive interactions to female intruders when their mate is removed during testing (Anzenberger, 1985; Evans, 1983). Thus, males respond differently dependent on their social situation.

Since male marmosets live in socially monogamous pair-bonded families, social conditions may affect how they respond to sexually relevant odors. We predict that social housing conditions will modulate the neuroendocrine and behavioral response a male shows to novel receptive females. For example, if male marmosets are living in social conditions where they are directly participating in parental care of infants, they might not be as responsive to novel sexually arousing signals because of their commitment to the successful rearing of their offspring. In contrast, males who are living without a mate would be expected to be very responsive to the odor of a receptive female since they would have fewer inhibiting factors controlling normal behavioral neuroendocrine responses and maybe more actively interested in females. Therefore, the behavioral responses of male marmosets introduced to novel females may depend upon whether their pair-bonded female is present at the time of exposure (Anzenberger, 1985; Evans, 1983). Males will display aggression towards females when their mate is present, but when the mate is absent, the male responds with sexual interest.

The objectives of this study were (1) to determine if ovulatory scents will elicit sexual behavior and acute hormonal responses from male common marmosets when presented with ovulatory odors from novel females in their home cages (Study 1), (2) to determine if the testosterone response is enhanced with controlled presentation (Study 2), and (3) to compare social housing condition of males with their endocrine response (Study 2). We measured serum cortisol and testosterone to determine if a gonadal activation would occur with or without an adrenal stress response to the testing conditions.

## Materials and methods

### *Subjects and housing*

A total of 30 male common marmosets were used to test olfactory cues and endocrine responses. During the study,

all males were housed in the Marmoset Colony of the National Primate Research Center—University of Wisconsin-Madison. Colony and husbandry conditions have been described elsewhere (Saltzman et al., 1994). Marmosets were kept under 12-h light cycles (6:30–18:30). Cage size for the families, paired, and single marmosets in the study were 0.6 × 0.91 × 1.83 m. Marmosets were housed in rooms with multiple cages and had visual access to other groups. All males were housed in one of three conditions: (1) the breeding male of a family, (2) paired with a female, or (3) as a single male who either had been removed from their families as an adult offspring due to fighting or who had lost a mate and was in transition before repairing. All males in the study had prior sexual experience with a female except male 723 who had been housed with siblings before the study. Housing conditions and scent exposure procedures were approved by the Graduate School Animal Care and Use Committee at the University of Wisconsin-Madison. The 15 males in Study 1 were 2.3 to 8 years of age and were living in families, as paired or as single males. For Study 2, Table 1 lists the age and social conditions for the 15 males.

### *Collection of ovulatory scent marks*

Females selected for scent mark collection were unfamiliar to all the males being tested. The selected females were administered 0.75 to 0.1 μg prostaglandin F2α analogue (cloprostenol sodium) in order to synchronize their timing of ovulation by terminating any conceptions or luteal cycles. Eight to ten days following prostaglandin treatment, females will ovulate. Scent samples were collected during this ovulatory period, and the females were bled to check progesterone levels, verifying that ovulation occurred. When progesterone values were 1.5 times the mean of preceding baseline values, ovulation had occurred (Harlow et al., 1984). Since not all females were bled daily for progesterone values, we selected scent samples that were collected within a 3-day period prior to the estimated day of ovulation and termed these the "ovulatory" period. No scent samples collected after progesterone values had elevated were used.

Table 1
Age and social status of male marmosets tested for response to scent marks

| Male | Age (years) | Social condition | Duration of condition |
|---|---|---|---|
| 523 | 5.9 | family | 32 months |
| 805 | 2.0 | family | 11 months |
| 489 | 6.4 | family | 48 months |
| 347 | 9.2 | family | 34 months |
| 545 | 5.7 | paired | 2 months |
| 861 | 2.3 | paired | 1 months |
| 679 | 4.1 | paired | 1 months |
| 835 | 3.3 | paired | 24 months |
| 857 | 2.8 | paired | 15 months |
| 963 | 1.4 | single | 5 days |
| 769 | 2.2 | single | 5 days |
| 723 | 2.6 | single | 5 days[a] |
| 881 | 1.2 | single | 12 days |
| 617 | 4.6 | single | 5 days |
| 775 | 3.6 | single | 7 days |

[a] Male 723 was removed from family with siblings for 5 days prior to our use.

Scent samples were collected from the females using the methods reported in Ferris et al. (2004). Females were presented with a raised reagent stopper made of ground glass where they would rub their anogenital area over the surface of the stopper, releasing scent secretions from the suprapubic and anogenital scent glands onto the glass. Secretions were usually mixed with small amounts of urine that were released during the rubbing. Once the female had marked the glass, it was removed from the cage and washed with 300 μl of deoxygenated purified water–ethanol (50:50). The solution was then transferred to a minivial for storage and frozen at −70°C. After determining which scents were ovulatory by progesterone values, the scents were mixed together and stored in aliquots which allowed for the use of a freshly thawed scent for each male. The vehicle control consisted of the same water–ethanol solution. All males in Study 1 received the same mixture of scents, and all males in Study 2 received a different mixture of scents from a second group of females.

### *Presentation of scents*

The scent secretions were presented to the males on a 2.5-cm-diameter wooden disk. One hundred microliters of either the ovulatory scent or the vehicle was applied to the disk using a pipette. A wooden substrate was chosen to mimic exposure to normal scent marks in the wild (Albone, 1984). Under both captive and natural conditions, female marmosets gouge holes into wood branches, tree trunks, or perches and then rub their anogential area onto the rough surface (Lazaro-Perea et al., 1999). Scent samples were presented to the males in a crossover design where half of the males would receive the vehicle first and the other half would receive the ovulatory scent first. Presentation occurred at the same time of day for 2 consecutive days (between 15:00 and 17:00) to control for possible diurnal variation in hormonal levels.

### *Predicting the testosterone response*

We bled the males 30 min from the onset of scent presentation based on the hormone responses recorded for those species referenced in the Introduction. Additionally, this time was selected based on a testosterone response within 1 h to hCG stimulation in male marmosets (Kholkute et al., 1983). Males were bled by quickly removing them from their cage or nest box and placing them into a restraint tube. The males were secured with velcro, while a 0.2-ml

blood sample from the femoral vein was collected. This process takes less than 5 min to complete, and the males were rewarded with a liquid treat. We performed two studies.

*Study 1. Scent testing in the home cage for behavioral and hormonal responses to a novel ovulatory scent*

Fifteen males (five family, five paired, five single) were tested for their response to scent marks from novel ovulatory females versus a vehicle control. Prior to testing the male, all cagemates were removed and placed in another room, leaving only the male. Behavioral observations were scored for 10 min starting at the time the wooden disk was set in the male's cage. Disks were placed next to the food bowl on a frequently used platform. Males were able to interact freely with the scent on the disk or to move about the cage. Since the disk is small and light, males frequently picked up the disk in their hands and manipulated it or they would put the disk in their mouth and move about the cage. Males were measured on their time to approach the disk, frequency of sniffing, licking, scent marking (both in the cage or on the disk), and touching the disk and duration of erections. Erections were recorded only if the male was positioned to expose the genital area, and the penis was erect. Total duration (in seconds) of the erect penis per male was averaged for the 10-min session. After 10 min, the disk was removed. Males were left alone until 30 min following initial placement of the disk into the cage. They were then removed from their cage and taken to another room to obtain a blood sample. Blood samples were collected without anesthesia and within 5 min from the time the males were caught. After blood collection, the males were provided with a treat, then he and his cagemates were returned to their home cage.

Observed behaviors were summarized for each male and averaged by condition. Differences between behavioral responses to the ovulatory scent and vehicle were tested by Wilcoxon signed ranks test due to the non-normal distribution of the data. We predicted that testosterone would be significantly higher with the ovulatory odors and cortisol levels would not differ. These were determined by pairwise Student $t$ test, one-tailed, $\alpha = 0.05$.

*Study 2. Scent testing under confinement for hormonal response*

To determine the hormonal response when males were in close proximity to the scent for the entire 10 min, we tested each male with the scent disk in a clean nest box. These are sleeping boxes used in each marmoset cage, and they measure 30 L × 21 W × 18 H cm with a series of round openings on one side for air movement. Each male was gently persuaded to enter the sleeping box in his home cage, and a door was pushed shut. The cage was removed to the anteroom where the male was transferred to a clean box for testing. We used different anterooms for the vehicle and ovulatory scent to prevent any cross-contamination of odors. A clean wooden disk was inserted into a cardboard cone, and the scent sample or vehicle was applied. The cone was inserted into one side of the sleeping box and held within 2 to 3 in. from the male's nose for 10 min. Since the enclosures were small, males were always within close contact with the scent disk. After this time, the disk was removed, and the males remained in the confined box until 30 min following the onset of scent presentation. Males were then removed from the box and immediately bled. All tests and bleeding occurred between 15:00 and 17:00 h. We predicted that testosterone levels would be higher with the presentation of the ovulatory scent but that cortisol would not differ. These were determined by pairwise Student $t$ test, one-tailed, $\alpha = 0.05$.

Additionally, significant differences in the percent change in hormone levels from vehicle to ovulatory scent for each male and social condition were determined by Kruskal–Wallis ANOVA with post hoc analysis by Mann–Whitney $U$ tests for unequal sample size. Correlation between the age of the male and his testosterone or cortisol response to periovulatory scent was determined by Pearson's product–moment correlation.

*Hormonal analysis*

Collected blood samples were stored on ice in heparanized syringes until all sampling had been completed for the afternoon. Samples were spun for 10 min, and the plasma removed and stored at −20°C until analysis. Each plasma sample was assayed to determine testosterone and cortisol levels.

Testosterone levels were determined using an enzyme-immunoassay modified from the methods described by Munro and Stabenfeldt (1984). Thawed plasma (10 μl) was extracted with 5 ml of ethyl ether, dried, and reconstituted in 300 μl of assay buffer with testosterone horseradish peroxidase at 1:100,000. This assay provides for a 2-h incubation with the testosterone antibody prior to substrate addition. The testosterone antibody (R156, Munro, University of California, Davis; 1:35,000) cross-reacted (50% binding) with the following substances: 92.4% with DHT, 11.2% with 4-androsten 3β, 17β-diol, 5.4% dehydroandrosterone, 3.4% androstanediol, 2.1% androstenedione, 0.5% androsterone, 0.4% epiandrosterone, 0.2% dehydroepiandrosterone, and less than 0.07% with other steroids. Testosterone concentration was determined by absorbance at 420 nm. Marmoset plasma samples were parallel to the testosterone standards (slopes did not differ, $t_{32} = 0.94$, $P > 0.05$), and accuracy was measured at each standard curve point ($N = 8$) was 98.60% ± 1.7% (mean ± SEM). Intra- and interassay coefficients of variation were 3.21 and 12.03, respectively.

Cortisol levels were determined using an $I^{125}$ radioimmunoassay developed and reported by Saltzman et al.

(1994). Marmoset plasma (4 μl) was used in the assay without the need for extraction. Intra- and interassay coefficients of variation were 5.75 and 10.67, respectively.

## Results

### *Study 1*

Behavioral responses to the ovulatory and vehicle scents during a 10-min presentation differed between conditions (Fig. 1). Frequency of sniffs ($Z$ = 2.19, $P$ = 0.029) and duration of erections ($Z$ = 2.37, $P$ = 0.018) were significantly greater for the ovulatory scent than for the vehicle. Other behaviors showed a trend towards increased frequency of licks and touch, or decreased latency to first sniff with the ovulatory scent. Testosterone levels were significantly higher for males after interacting with the ovulatory scent disk than with the vehicle scent ($T$ = 2.12, $P$ = 0.03), but there were no differences between the cortisol response (Fig. 2).

No correlation between specific behaviors corresponded to levels of testosterone or percent change. Additionally, males who responded to the ovulatory scent disk with the most behaviors did not necessarily show the highest testosterone levels.

### *Study 2*

As with the first study, testosterone levels were significantly higher with the ovulatory scent than the vehicle (mean, vehicle: 11.41 ± 1.94, periovulatory: 16.91 ± 2.85; $T$ = 2.05, $P$ = 0.029). There were no significant differences in cortisol by condition. All cortisol levels under confinement conditions were well within the range of normal nonmanipulated male afternoon cortisol levels (27.6–361.7 μg/dl, mean = 154.5, $n$ = 10).

When males were partitioned by their social condition (Fig. 3), there were significant differences in testosterone.

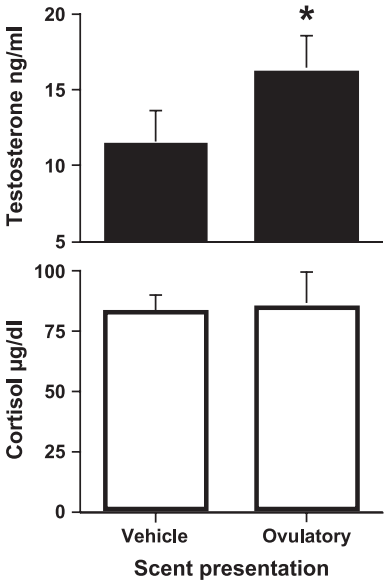


Fig. 2. Mean (± SEM) plasma testosterone and cortisol responses in 15 male common marmosets to female ovulatory scent marks and vehicle control presented on a wooden disk. Males were tested for 10 min in their home cage, and a blood sample was taken 30 min following the beginning of the presentation. *Significantly different from vehicle, paired Student $t$ test, testosterone, $P$ = 0.03.

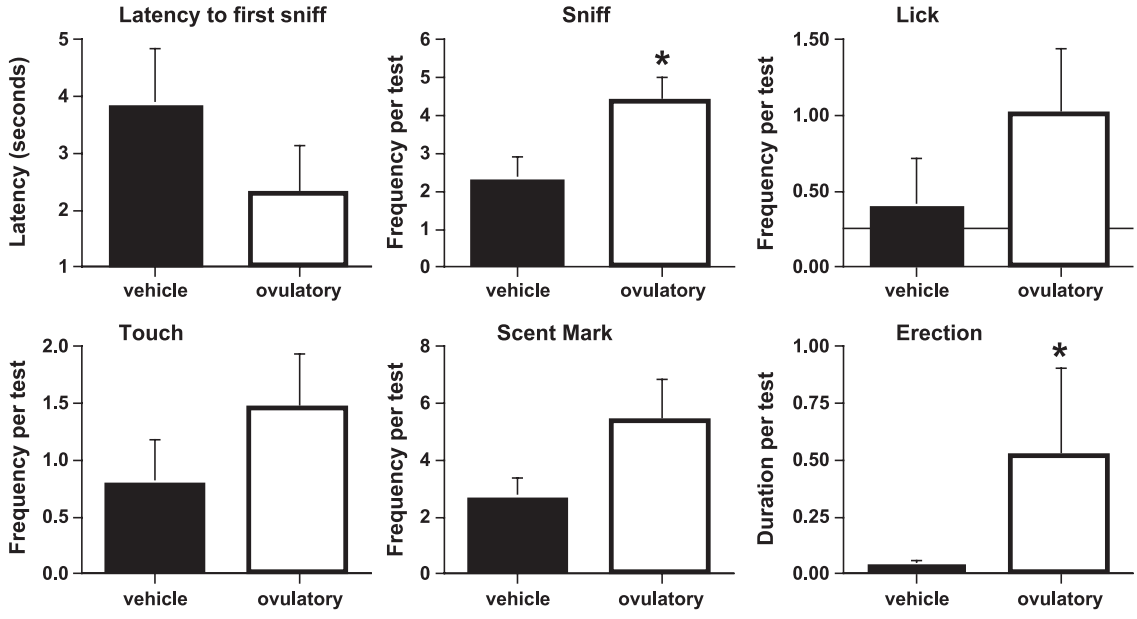


Fig. 1. Mean (± SEM) behavioral responses in 15 male common marmosets to female ovulatory scent secretions and vehicle control presented on a wooden disk. Males were tested for ten minutes in their home cage in the absence of other group members. *Significantly different from vehicle, $P$ < 0.05, Wilcoxon signed ranks test. Duration of erections were recorded in minutes.

Significant increases in testosterone occurred for paired males ($t = 2.89$, $P = 0.02$) and single males ($t = 1.97$, $P = 0.05$) with the ovulatory scent, but no differences were found for family males. Cortisol levels were not significantly different for the males in any social condition.

The same results were found when the percent increase in testosterone and cortisol from vehicle to periovulatory scent was determined. There were significant differences between the three social conditions (KW = 7.52, $P = 0.02$). Family-living males showed a significantly lower percent change to ovulatory odors from vehicle levels than did the paired males ($U = 0$, $N = 4,5$, $P = 0.008$) or the single males ($U = 3$, $N = 4,6$, $P = 0.03$): mean percent increase for family males = 82.78 ± 21.50, paired males = 135.35 ± 9.16, and single males = 368.90 ± 161.4. Single males showed the highest change from vehicle to ovulatory and differed statistically from paired males ($U = 5$, $N = 6,5$, $P = 0.04$). Single males had lower testosterone levels than other males during the vehicle stimulus, but this was not significantly different.

Changes in cortisol levels by social condition were not significant. Family males did not differ significantly from paired males or from single males, and paired males did not differ from single males. No significant correlation was found between age of the male and testosterone or age and cortisol. No significant correlation was found for duration of social condition and percent change in testosterone or cortisol.

## Discussion

Male common marmosets showed both behavioral and endocrine responses indicative of sexual arousal to novel female scent marks. These results are important for several reasons: (1) in the socially monogamous common marmoset, social housing condition influences a male's hormonal and behavioral response to sexually arousing cues, (2) ovulatory cues from scent secretions alone can act as an arousal cue to stimulate both behavioral and gonadal responses from a male primate, and (3) male primates show the same rapid increase of testosterone as do other mammals, that is, within 30 min.

We suggest that male marmosets have a natural behavioral and neuroendocrine response to sexually relevant cues such as ovulatory scent marks from novel females. However, under stable family conditions, there may be an inhibitory process that prevents males from exhibiting a full response. This could be part of their monogamous social system. The family males in this study were directly involved in infant care at the time of testing. Single males, such as those in the present experiment, would have the best opportunity to show a high testosterone response to sexually relevant cues from a novel female. While single housing is not considered a normal condition for male marmosets, there are times when nonparental wild marmosets are assessing other groups to determine the reproductive state of females as potential pairmates (Lazaro-Perea, 2001). A

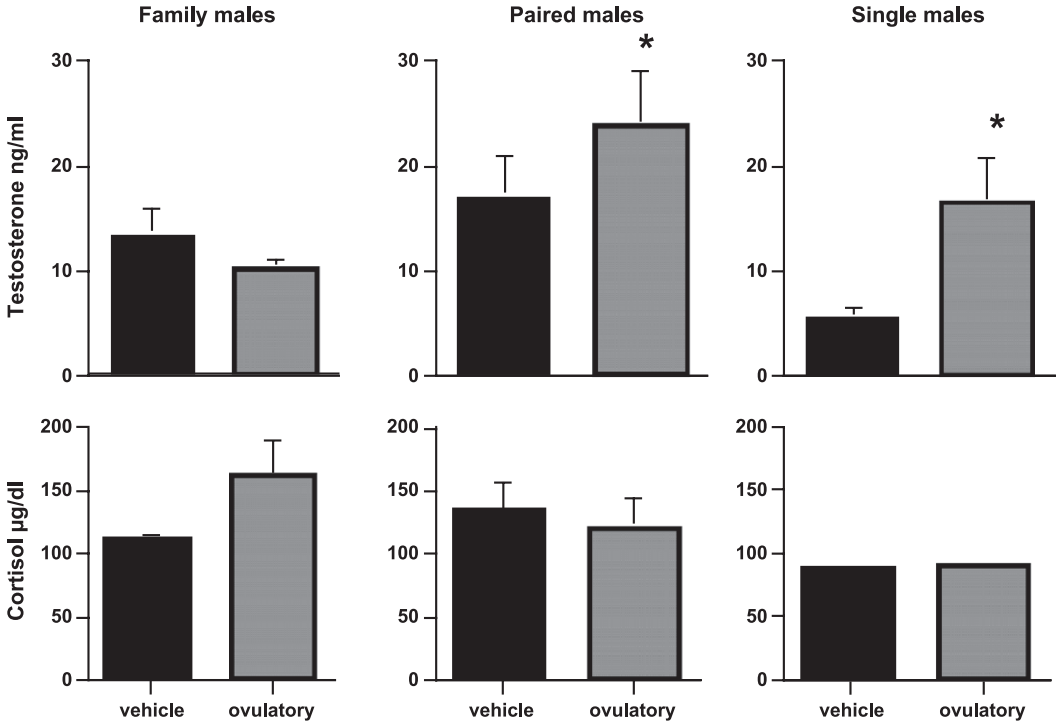


Fig. 3. Mean (± SEM) testosterone and cortisol levels in response to vehicle and ovulatory scent by social condition with sustained scent presentation for 10 min. Levels of testosterone were significantly higher with the ovulatory scent for paired ($P = 0.02$) and single males ($P = 0.05$), paired Student $t$ test. There was no significant difference in cortisol levels between vehicle and ovulatory scents for males under any condition.

male's testosterone response to a novel sexually relevant stimulus might be a method to test a male's pair-bond strength to his family or pairmate.

An alternative explanation for the significant differences between testosterone by social conditions would be that mated males are continually receiving sexual cues and producing a testosterone response, and therefore, they may already have elevated levels of testosterone. Family and paired males on average had higher levels of testosterone during the vehicle test than did the single males, but this was not significantly different. However, this may be expected as the family and paired males were living with females that were in early pregnancy or in the luteal phase of their cycle and therefore should not be producing receptive or arousal signals. These males may have learned to associate cues indicative of ovulation with the individual specific cues from their mate and may ignore cues from novel females. Captive paired males in this colony are typically living with a mate where pregnancies are terminated for management reasons. The lack of successful reproduction may therefore lead paired males to be more interested in odors of novel females than family males. Another explanation may be that most of the paired males in the current study had been with their mates only a few months and may not yet have formed a strong pair bond.

The present study extends our knowledge of how sexual cues are processed by male marmosets to show that social cues will also stimulate the endocrine system. Males in Study 2 were in constant exposure to the ovulatory scent as were the male marmosets in our fMRI paradigm providing confirmation of the brain imaging activation patterns found for males in the imaging studies (Ferris et al., 2001, 2004). The position and duration of the scent exposure were the same as the fMRI studies, and marmosets showed a robust testosterone response to the ovulatory scent, except for the family males. While this method of presentation does not allow for examining a behavioral response to the novel ovulatory cues, it does provide for the optimum reception of the olfactory cues from the scent secretions.

Male marmosets have enhanced brain activity in areas known to be involved in sexual behavioral responses, that is, the MPOA/AH. Studies in rodents demonstrate that input from accessory olfactory bulb and vomeronasal systems increase neuronal firing when males are exposed to females (Luo et al., 2003). Toxic or electrolytic lesioning of the MPOA/AH disrupts male sexual behavior (Kindon et al., 1996; Paredes and Baum, 1995) which is reversible with fetal hypothalamic transplant in adult males (Paredes et al., 1993). The olfactory and chemical system, as well as other sensory systems, connects to the preoptic area of the hypothalamus (Everitt, 1990; see Dixson, 1998). This is a part of the vomeronasal projection pathway, which is involved in the processing of chemosensory sexually relevant cues (Paredes, 2003) and known to be involved in male sexual behavioral responses to female arousal cues (Dixson and Lloyd, 1989). Activation of the MPOA stimulates the release of LHRH (Everett, 1988). This appears to be an immediate response from the brain with pituitary release of LH activating testosterone release from the testes. Male marmosets are known to respond to LHRH and hCG within 2 h (Kholkute et al., 1983; Lunn et al., 1990), but no one has yet reported a testosterone response in the marmoset as early as 30 min. Although we did not do serial time sampling to determine the optimal timing for testosterone response, we did show a response by 30 min as found in other male mammals.

In Study 1, we were able to examine the behavioral response to the scent secretions but do not ensure that males had optimum exposure to the novel scent. Only two behaviors, "sniff" and "erection," differed significantly between the vehicle and ovulatory scent conditions. That increased erection time was significant indicates that the ovulatory odors were indeed sexually arousing. Changes in the behaviors that were not significant were all in the expected direction, and frequency of licks and scent marking was very close to significance. A stronger behavioral response might have occurred if we could have ensured that the males remained in contact with the scent for the entire 10 min such as what occurred in Study 2. Some males repeatedly produced long calls during the testing session which are associated with locating group members which indicates that they may have been distressed. Alternatively, if males had been habituated to having cagemates removed prior to testing or if we had left paired females in the cage during testing as has been reported for cottontop tamarins (Ziegler et al., 1993), males might have been less distracted. Male cottontop tamarins tested with novel ovulatory scents while their pair-bonded female remains in the cage responded with significant increases in mounting their female mate and also with increased duration of erections. If the signals produced by the novel ovulatory scent mediate arousal responses in males, then a normal response should include sexual responses to the pair-bonded female if males are tested with their mates present. While most male marmosets spent time interacting with the ovulatory scent, not all males even approached the disk. In male European starlings (*Sturnus vulgarus*) that have been tested with female presence during the breeding season, not all males showed a behavioral response to the presence of a female, but all showed a testosterone response (Pinxten et al., 2003). For the birds showing a behavioral response to the female, they also had significantly higher levels of testosterone than males showing no behavioral response. While we did not find this in our study, a connection between the hormonal and behavioral response would be expected.

Whether social conditions could modulate the timing of the testosterone response is unknown, but it appears that the social conditions studied here are highly important for behavioral endocrine responses in primates. It is important to note that the social housing condition had no effect on cortisol levels, which remained within normal afternoon

levels for unmanipulated males even in the confinement study.

These data lead us to question how social life changes can affect how males respond hormonally. For instance, since males show differences in testosterone responsiveness dependent upon their social condition, we would predict that individual males going from bachelorhood to fatherhood should show a reduction in testosterone responsiveness to novel ovulatory cues. As a male's social life changes, so will its brain responsiveness. Pair-bonded males with dependent offspring appear to be the least responsive to novel ovulatory cues which may indicate that cortical stimulation inhibits the biological hormonal responsiveness. Family males have invested more in their association with the breeding female and in assuring the successful rearing of their offspring. We hope to address these issues in the future.

## Acknowledgments

We thank Fritz Wagner, Dan Wittwer, and Steve Jacoris for assistance with hormonal analyses and Rhoda Kapugi, Megan Sosa, and Jennifer Lambert-Newman for help with the marmosets. Dr. R. Almond provided helpful comments on the revision of the manuscript. Parts of this research were presented at the American Society of Primatology Meeting, July 2003. This work was supported by grants from the National Institutes of Health MH 58700 to C.F. Ferris, MH35215 to C.T. Snowdon and T.E. Ziegler, and RR00167 to the National Primate Research Center, University of Wisconsin-Madison.

## References

Albone, E.S., 1984. Mammalian Semiocemistry: The Investigation of Chemical Signals between Mammals. John Wiley & Sons Ltd, Chichester.

Anzenberger, G., 1985. How stranger encounters of common marmosets (*Callithrix jacchus jacchus*) are influenced by family members: the quality of behavior. Folia Primatol. 45, 204–224.

Beach, F.A., 1956. Characteristics of masculine "sex drive". In: Jones, M.R. (Ed.), Nebraska Symposium on Motivation. University of Nebraska Press, Lincoln, pp. 1–31.

Bronson, F.H., Desjardins, C., 1982. Endocrine responses to sexual arousal in male mice. Endocrine 111, 1286–1291.

Carani, C., Bancroft, J., Del Rio, G., Granata, A.R.M., Facchinetti, F., Marrama, P., 1990. The endocrine effects of visual erotic stimuli in normal men. Psychoneuroendo 15, 207–216.

Digby, L.J., 1995. Social organization in a wild population of *Callithrix jacchus*: II. Intragroup social behavior. Primates 36, 361–375.

Dixson, A.F., 1998. Primate Sexuality: Comparative Studies of the Prosimians, Monkeys, Apes, and Human Beings. Oxford Univ. Press, Oxford.

Dixson, A.F., Lloyd, S.A.C., 1989. Effects of male partners upon proceptivity in ovariectomized estradiol-treated marmosets (*Callithrix jacchus*). Horm. Behav. 23, 211–220.

Ellendorff, R., Parvizi, N., Pomerantz, D.K., Hartjen, A., Konig, A., Smidt, D., Elsaesser, F., 1975. Plasma luteinizing hormone and testosterone in the adult male pig: 24 hour fluctuations and the effect of copulation. J. Endocrinol. 67, 403–410.

Elvira, M.C.R., Herndon, J.G., Wilson, M.E., 1982. Influence of estrogen-treated females on sexual behavior and male testosterone levels of a social group of rhesus monkeys during the nonbreeding season. Biol. Reprod. 26, 825–834.

Evans, S., 1983. The pair-bond of the common marmoset, *Callithrix jacchus*: an experimental investigation. Anim. Behav. 31, 651–658.

Everett, J.W., 1988. Pituitary and hypothalamus: perspectives and overview. In: Knobil, E., Neill, J. (Eds.), The Physiology of Reproduction. Raven Press Ltd., New York, pp. 1143–1159.

Everitt, B.J., 1990. Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci. Biobehav. Rev. 14, 217–232.

Ferris, C.F., Snowdon, C.T., King, J.A., Duong, T.Q., Ziegler, T.E., Ugurbil, K., Olson, D.P., Ludwig, R., Schultz-Darken, N.J., Wu, Z., Sullivan Jr., J.M., Tannenbaum, P.L., Vaughan, J.T., 2001. Functional imaging of brain activity in conscious monkeys responding to sexually arousing cues. NeuroReport 12, 2–7.

Ferris, C.F., Snowdon, C.T., King, J.A., Sullivan Jr., J.M., Ziegler, T.E., Olson, D.P., Schultz-Darken, N.J., Tannenbaum, P.L., Ludwig, T., Wu, Z., Einspanier, A., Vaughan, J.T., Duong, T.Q., 2004. Activation of neural pathways associated with sexual arousal in non-human primates. J. Magn. Reson. Imag. 19, 168–175.

Gordon, T.P., Bernstein, I.S., Rose, R.M., 1978. Social and seasonal influences on testosterone secretion in the male rhesus monkey. Physiol. Behav. 21, 623–627.

Harlow, C.R., Hearn, J.P., Hodges, J.K., 1984. Ovulation in the marmoset monkey: endocrinology, prediction and detection. J. Endocrinol. 103, 17–24.

Harrison, M.L., Tardif, S.D., 1989. Species differences in response to conspecific intruders in *Callithrix jacchus* and *Saguinus oedipus*. Int. J. Primatol. 10, 343–362.

Katongole, C.B., Naftolin, F., Short, R.V., 1971. Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. J. Endocrinol. 50, 457–466.

Kendrick, K.M., Dixson, A.F., 1983. The effect of the ovarian cycle on the sexual behaviour of the common marmoset (*Callithrix jacchus*). Physiol. Behav. 30, 735–742.

Kholkute, S.D., Aitken, R.J., Lunn, S.F., 1983. Plasma testosterone response to hCG stimulation in the male marmoset monkey (*Callithrix jacchus jacchus*). Reprod. Fertil. 67, 457–463.

Kindon, H.A., Baum, M.J., Paredes, R.J., 1996. Medial preoptic/anterior hypothalamic lesions induce a female-typical profile of sexual partner preference in male ferrets. Horm. Behav. 30, 514–527.

Kruger, T., Exton, M.S., Pawlak, C., von zur Muhlen, A., Hartmann, U., Schedlowski, M., 1998. Neuroendocrine and cardiovascular response to sexual arousal and orgasm in men. Psychoneuroendocrine 23, 401–411.

Lazaro-Perea, C., 2001. Intergroup interactions in wild common marmosets, *Callithrix jacchus*: territorial defense and assessment of neighbors. Anim. Behav. 62, 11–21.

Lazaro-Perea, C., Snowdon, C.T., Arruda, M.F., 1999. Scent-marking behavior in wild groups of common marmosets (*Callithrix jacchus*). Behav. Ecol. Sociobiol. 46, 313–324.

Lloyd, S.A.C., Dixson, A.F., 1988. Effects of hypothalamic lesions upon the sexual and social behaviour of the male common marmoset (*Callithrix jacchus*). Brain Res. 463, 317–329.

Lunn, S.F., Dixson, A.F., Sandow, J., Fraser, H.M., 1990. Pituitary-testicular function is suppressed by an LHRH antagonist but not by an LHRH agonist in the marmoset monkey. J. Endocrinol. 125, 233–239.

Luo, M., Fee, M.S., Katz, L.C., 2003. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. Science 299, 1196–1201.

Macrides, R., Bartke, A., Fernandez, F., D'Angelo, W., 1974. Effects of exposure to vaginal odor and receptive females on plasma testosterone in the male hamster. Neuroendocrinology 15, 355–364.

Munro, C., Stabenfeldt, F., 1984. Development of microtiter plate enzyme immunoassay for the determination of progesterone. J. Endocrinol. 101, 41–49.

Paredes, R.G., 2003. Medial preoptic area/anterior hypothalamus and sexual motivation. Scand. J. Psychol. 44, 203–212.

Paredes, R.G., Baum, M.J., 1995. Altered sexual partner preference in male ferrets given excitotoxic lesions of the preoptic area/anterior hypothalamus. J. Neurosci. 15, 5530–6619.

Paredes, R.G., Pina, A.L., Bermudez-Rattoni, F., 1993. Hypothalamic but not cortical grafts induce recovery of sexual behavior and connectivity in medial preoptic area-lesioned rats. Brain Res. 620, 351–355.

Perret, M., Schilling, A., 1995. Sexual responses to urinary chemosignals depend on photoperiod in a male primate. Physiol. Behav. 58, 633–639.

Pinxten, R., Ridder, E., Eens, M., 2003. Female presence affects male behavior and testosterone levels in the European starling (*Sturnus vulgaris*). Horm. Behav. 44, 103–109.

Rose, R.M., Gordon, T.P., Bernstein, I.S., 1972. Plasma testosterone levels in the male rhesus: influences of sexual and social stimuli. Science 172, 643–645.

Saginor, M., Horton, R., 1968. Reflex release of gonadotropin and increased plasma testosterone concentration in male rabbits during copulation. Endocrine 82, 627–630.

Saltzman, W., Schultz-Darken, N., Scheffler, G., Wegner, F., Abbott, D., 1994. Social and reproductive influences on plasma cortisol in female marmoset monkeys. Physiol. Behav. 56, 801–810.

Smith, T.E., Abbott, D.H., 1998. Behavioral discrimination between circumgenital odor from peri-ovulatory dominant and anovulatory female common marmosets (*Callithrix jacchus*). Am. J. Primatol. 46, 265–284.

Stevenson, M.F., Rylands, A.B., 1988. The marmosets, genus *Callithrix*. In: Mittermeier, R.A., Rylands, A.B., Coimbra-Filho, A.F., da Fonseca, G.A.B. (Eds.), Ecology and Behavior of Neotropical Primates. World Wildlife Fund, Washington, DC, pp. 131–222.

Stoleru, S.G., Ennaji, A., Cournot, A., Spira, A., 1993. LH pulsatile secretion and testosterone blood levels are influenced by sexual arousal in human males. Psychoneuroendocrinology 18, 205–218.

Ziegler, T.E., Epple, G., Snowdon, C.T., Porter, T.A., Belcher, A.M., Kuderling, I., 1993. Detection of the chemical signals of ovulation in the cotton-top tamarin, *Saguinus oedipus*. Anim. Behav. 45, 313–322.