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Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen

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Although high testosterone (T) levels inhibit paternal behaviour in birds breeding in temperate zones many paternal mammals have a very different breeding biology, characterized by a post-partum oestrus. In species with post-partum oestrus, males may engage in T-dependent behaviours such as aggression and copulation simultaneously with paternal behaviour. We previously found that T promotes paternal behaviour in the California mouse, *Peromyscus californicus*. We examine whether this effect is mediated by the conversion of T to oestradiol (E_2) by aromatase. In the first experiment, gonadectomized males treated with T or E_2 implants showed higher levels of huddling and pup grooming behaviour than gonadectomized males treated with dihydrotestosterone or empty implants. In the second experiment, we used an aromatase inhibitor (fadrozole) (FAD) to confirm these results. Gonadectomized males treated with T + vehicle or E_2 + FAD showed higher levels of huddling and pup grooming behaviour than gonadectomized males treated with T + FAD or empty implants. Although E_2 is known to promote the onset of maternal behaviour to our knowledge our results are the first to demonstrate that E_2 can promote paternal behaviour in a paternal mammal. These results may explain how mammals express paternal behaviour while T levels are elevated.

Keywords: testosterone; oestrogen; paternal behaviour; aromatase

1. INTRODUCTION

Testosterone (T) is known to promote a wide array of behaviours associated with reproduction, including intermale competition (Monaghan & Glickman 1992), courtship behaviour (Adkins-Regan 1998) and mating behaviour (Meisel & Sachs 1994). In a number of mammalian species, male T levels decline after the birth of offspring (Brown *et al.* 1995; Reburn & Wynne-Edwards 1999; Nunes *et al.* 2001). These findings have usually been interpreted as evidence that T has a negative effect on male parental behaviour. This generalization is largely derived from the literature examining how high T levels mediate a life-history trade-off in temperate zone birds by promoting mating behaviour while inhibiting paternal behaviour (Hegner & Wingfield 1987; Ketterson *et al.* 1992). In temperate zone birds, mate acquisition usually occurs early in the breeding season when T levels are high, and paternal behaviour occurs later when T levels are low (Ball 1992). Despite intensive study of the effects of high T levels on paternal behaviour, relatively little is known about how baseline T levels affect paternal behaviour. Testosterone replacement treatment in gonadectomized ring doves enhances the positive effects of progesterone on paternal behaviour (Stern & Lehrman 1969). This suggests that there may be some variability in how T affects paternal behaviour. Correlational and experimental studies in mammals also suggest that T does not always decrease paternal behaviour.

Male parental behaviour is present in a number of rodent, canid and primate species (Woodroffe & Vincent 1994). The breeding biology of many mammals is characterized by a post-partum oestrus (common marmoset, McNeilly *et al.* (1981); cotton-top tamarin, Ziegler *et al.* (1987); Djungarian hamster, Roy & Wynne-Edwards (1995); Siberian hamster, Parkening & Collins (1991)), such that females are fertile shortly after parturition. In contrast to temperate zone birds, paternal mammals often engage in paternal, mating and possibly mate guarding behaviour simultaneously. These demands may be reflected in studies that indicate that T does not consistently inhibit mammalian paternal behaviour. Male T levels are high at parturition in the monogamous Djungarian hamster and decline shortly thereafter (Reburn & Wynne-Edwards 1999). In biparental Mongolian gerbils, male androgen levels increase at parturition and decline to baseline levels after three days (Brown *et al.* 1995). These transient increases in hamster and gerbil androgens are qualitatively similar to a transient decrease in T levels after parturition seen in some human fathers (Storey *et al.* 2000; Berg & Wynn-Edwards 2001). In cotton-top tamarins, male urinary T levels increase throughout pregnancy and remain elevated after parturition (Ziegler & Snowdon 2000). Male Djungarian hamsters (Jones & Wynn-Edwards 2000) and cotton-top tamarins (Ziegler & Snowdon 2000) show high levels of paternal behaviour immediately after parturition, despite high T levels. Experimental manipulations of T have yielded mixed results. Gonadectomy increases paternal behaviour in male Mongolian gerbils (Clark & Galef 1999). However, the effect of gonadectomy on paternal behaviour in prairie voles has been inconsistent (Wang & De Vries 1993; Lonstein & De Vries 1999). We recently reported that castration reduces, and castration combined with T-replacement therapy maintains, paternal behaviour in male California mice, *Peromyscus californicus* (Trainor & Marler 2001).

The California mouse is an attractive model for the

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study of the physiology of paternal behaviour. Males show high levels of paternal behaviour starting on the day of parturition (Gubernick & Alberts 1987), and continue to care for pups throughout their development (Bester-Meredith *et al.* 1999). Interestingly, the expression of paternal behaviour coincides with the post-partum oestrus (Gubernick 1988). Most males undergo a transition from attacking or ignoring pups to exhibiting paternal behaviour after the birth of their own pups (Gubernick & Nelson 1989). A DNA paternity study conducted in the field found no evidence for extra-pair young in the field (Ribble 1991), suggesting that males may guard their mates to prevent extra-pair fertilizations. Here we explore the mechanism(s) by which T promotes paternal behaviour in this biparental rodent.

Generally, T can regulate behaviour by binding directly to androgen receptors or through conversion to androgenic or oestrogenic metabolites. Testosterone can be converted to dihydrotestosterone (DHT), which then binds to androgen receptors. Aromatase converts T to oestradiol (E_2), which then binds to oestrogen receptors. Aromatase cannot convert DHT to a corresponding oestrogen. The effects of T on paternal behaviour in California mice could occur via androgens, oestrogens, or both. Oestradiol is an important steroid hormone promoting the onset of maternal behaviour in mammals (Bridges 1996). Intriguingly, gonadectomized adult male rats treated with E_2 and progesterone implants exhibited paternal behaviour when E_2 was infused into the medial preoptic area (Rosenblatt & Ceus 1998), even though this behaviour is not normally expressed. These results led us to hypothesize that T promotes paternal behaviour in California mice through conversion to E_2. We tested this hypothesis in two experiments. First, we conducted a traditional hormone manipulation study in which males were gonadectomized and given silastic implants containing T, DHT, E_2, or an empty implant. Second, we tested whether aromatase mediates the effects of T by using FAD, an effective aromatase inhibitor (Clancy & Michael 1994).

2. MATERIAL AND METHODS

(a) *Subjects*

We used reproductively experienced male California mice reared in a laboratory colony at the University of Wisconsin, Madison. Subjects were housed in male–female pairs in standard cages and were given Purina 5001 mouse chow and water *ad libitum*. Colony rooms were kept under a 13 L : 11 D cycle with lights on at 05.00. Behavioural observations were conducted during the dark phase between 19.00 and 21.00 under dim red light. Animals were maintained in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

(b) *Experiment I: dihydrotestosterone and oestradiol treatment*

We anaesthetized males with isoflurane and performed bilateral castrations through a single incision at the scrotum (Trainor & Marler 2001). Each male received a 10 mm long subcutaneous empty silastic implant (inner diameter 0.147 cm, outer diameter 0.196 cm) or an implant packed with 1 mm of T, DHT, or E_2. We randomly assigned 10 males to each group. Males were given one week of recovery in isolation and then returned to their mates. Two days prior to testing, males were placed in large Plexiglas observation cages. The cages contained a smaller chamber (22 cm × 29 cm × 30 cm) and a larger chamber (30 cm × 29 cm × 30 cm) fitted with a running wheel, food and a water bottle. While in the observation cage, each male was exposed to the soiled bedding of his mate. We assessed paternal behaviour by exposing each male to an unrelated 1–3 day old pup for 10 min at two and three weeks after treatment. These methods have been described in detail elsewhere and yield results that are equivalent to tests using a male's own pups (Trainor & Marler 2001). We recorded the amount of time males spent huddling with the pup and grooming the pup. Behavioural tests were scored by an observer blind to treatment assignments. Data were analysed using repeated-measures ANOVA. We tested two hypotheses with independent contrasts. First, if T promotes paternal behaviour via aromatization then males given T or E_2 implants should exhibit higher levels of paternal behaviour than males given DHT or empty implants (oestrogen hypothesis). Second, if T promotes paternal behaviour via activation of androgen receptors then males given T or DHT implants should exhibit higher levels of paternal behaviour than males given E_2 or empty implants (androgen hypothesis). *Post hoc* comparisons were made using Fisher's protected least significant difference (LSD).

(c) *Experiment II: fadrozole treatment*

All males were bilaterally castrated as described above. We randomly assigned 13 males to each of the following subcutaneous implant combinations: (i) T silastic implant and FAD implant [T + FAD]; (ii) T silastic implant and FAD vehicle implant [T + vehicle]; (iii) E_2 silastic implant and FAD implant [E_2 + FAD]; or (iv) empty silastic implant. FAD was dissolved in distilled water and administered with osmotic implants (Alzet model 2002) at a treatment of 0.25 mg kg^{-1}. This dose effectively inhibits aromatase both centrally and peripherally in rodents (Clancy & Michael 1994) and primates (Zumpe *et al.* 1993). After treatment, males were housed individually for the duration of the experiment, but were exposed to soiled bedding from their mates. Paternal behaviour was assessed as described in experiment I at 12 and 13 days after treatment. We chose this testing schedule because osmotic implants release their product for 14 days. Behavioural tests were scored by an observer blind to treatment assignments. Data were analysed using repeated-measures ANOVA. We tested two hypotheses with independent contrasts. First, if T promotes paternal behaviour through aromatization and activation of oestrogen receptors, then males treated with T + vehicle or E_2 + FAD should show more paternal behaviour than males treated with T + FAD or empty implants (oestrogen hypothesis). Second, if T promotes paternal behaviour through activation of androgen receptors then males treated with T + vehicle or T + FAD should show more paternal behaviour than males treated with E_2 + FAD or empty implants (androgen hypothesis).

(d) *Hormone measurements*

After behaviour tests all subjects were lightly anaesthetized with isoflurane and rapidly decapitated to collect trunk blood. Samples were centrifuged and plasma was collected and stored at −80 °C. In experiment II, we measured the residual fluid in osmotic implants to confirm product delivery. Samples were extracted twice with ethyl ether and steroid hormones were separated using celite chromatography. We measured T using an

enzyme immunoassay (EIA) previously validated for California mice (Trainor & Marler 2001). We measured DHT using an EIA (Ziegler *et al.* 2000), and E_2 using a radioimmunoassay (French *et al.* 1983). When assay concentrations for serial dilutions of a DHT-spiked California mouse plasma pool (100–0.8 µl, $n = 8$) and an E_2-spiked plasma pool (288–1.3 µl, $n = 8$) were compared to standards, computed regression lines did not differ in slope for DHT ($t_{28} = 0.72$, $p > 0.05$) or E_2 ($t_{28} = 1.32$, $p > 0.05$). We measured DHT using an EIA (Ziegler *et al.* 2000) and E_2 using a radioimmunoassay (RIA; French *et al.* 1983). Accuracies measured at each standard curve point of the DHT EIA (0.5–100 pg, 60 µl of plasma, $n = 8$) and E_2 RIA (3–256 pg, 200 µl of plasma, $n = 8$) were 105.43 ± 3.3% and 110.86 2.1%, respectively. We used different volumes of California mouse plasma pools to measure coefficients of variation at 80% binding and 50% binding on the standard curves for each hormone assay. The intrassay coefficients of variation were measured using pools of California mouse plasma and were 7.09%, 3.57%, and 7.1% for the T (four assays), DHT (one assay), and E_2 (two assays) assays. The interassay coefficients of variation were 14.82% and 20.99% for T and E_2, respectively. We used rank correlations to correlate T and DHT levels with behavioural data averaged across the two tests and one-way ANOVA to compare T levels in experiment II. For E_2, despite our use of very large sample volumes (300 µl), many of the readings (14 out of 23, 61%) were at the extreme lower end of the standard curve as defined by 90% binding. To test whether variations in E_2 levels were associated with variation in paternal behaviour, we used the median E_2 level within each experiment to divide the males into those with E_2 levels above the median (high E_2) and those with E_2 levels below the median (low E_2). For each experiment, we compared parental behaviours averaged across the two behaviour tests for high E_2 males and low E_2 males with a Mann–Whitney *U*-test.

3. RESULTS

(a) *Experiment I*

Overall, the results were consistent with the oestrogen hypothesis, but not the androgen hypothesis (figure 1). An independent contrast testing the oestrogen hypothesis indicated that males treated with T or E_2 showed significantly higher levels of huddling ($F_{1,36} = 12.04$, $p < 0.001$) and pup grooming behaviour ($F_{1,36} = 8.32$, $p = 0.007$) compared to males treated with DHT or empty implants. An independent contrast testing the androgen hypothesis indicated that males treated with T or DHT were not significantly different from males treated with E_2 or empty implants for huddling ($F_{1,36} = 0.16$, $p = 0.69$) or pup grooming behaviour ($F_{1,36} = 0.01$, $p = 0.98$). There was no difference in huddling ($F_{1,36} = 0.48$, $p = 0.49$) or pup grooming ($F_{1,36} = 0.09$, $p = 0.77$) across the first and the second behaviour tests. There was no test–treatment interaction for huddling ($F_{3,36} = 0.43$, $p = 0.73$) or pup grooming ($F_{3,36} = 0.84$, $p = 0.48$).

(b) *Experiment II*

These results were also consistent with the oestrogen hypothesis, but not the androgen hypothesis (figure 2). An independent contrast testing the oestrogen hypothesis indicated that males treated with T + vehicle or E_2 + FAD showed significantly higher levels of huddling ($F_{1,48} = 18.71$, $p < 0.001$) and pup grooming behaviour

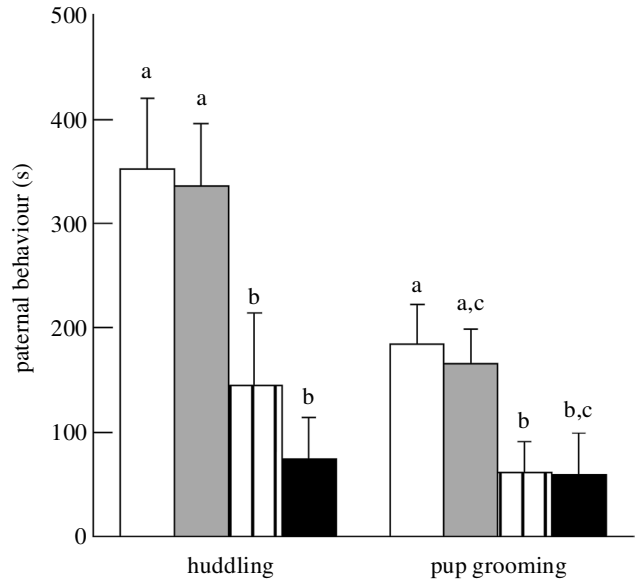

Figure 1. Paternal behaviour averaged across tests in gonadectomized males with different steroid hormone treatments in experiment I ($n = 10$ per group). Differences between groups are indicated by different letters above the bars (Fisher's LSD $p < 0.05$). There is a non-significant trend for males treated with E_2 to groom pups more than males treated with empty implants ($p = 0.05$). White bars, testosterone; grey bars, oestradiol; striped bars, DHT; black bars, empty.

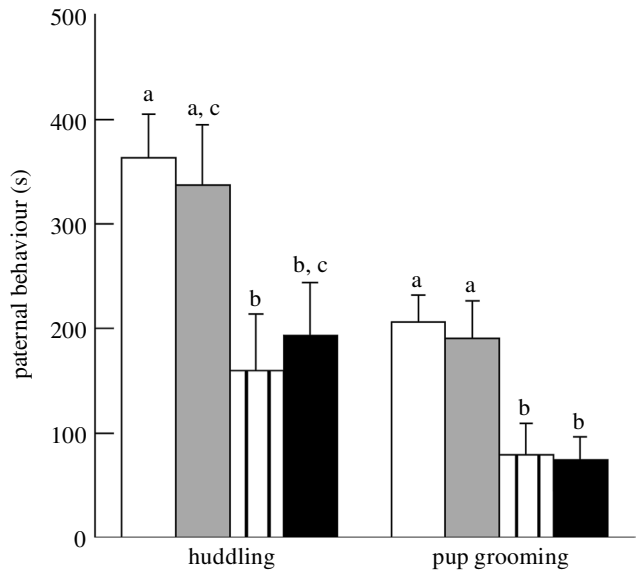

Figure 2. Paternal behaviour averaged across tests in gonadectomized males with different steroid hormone and aromatase inhibitor (FAD) treatments in experiment II ($n = 13$ per group). Differences between groups are indicated by different letters above the bars (Fisher's LSD $p < 0.05$). There is a non-significant trend for males treated with E_2 + FAD to huddle with pups more than males treated with empty implants ($p = 0.05$). White bars, T + vehicle; grey bars, E_2 + FAD; striped bars, T + FAD; black bars, empty.

($F_{1,48} = 11.37$, $p = 0.001$) compared to males treated with T + FAD or empty implants. An independent contrast testing the androgen hypothesis indicated that males

Table 1. Oestradiol levels and median huddling and pup grooming for experiments I and II. Males in each experiment divided into high E_2 and low E_2 males based on the overall median.[a]

	experiment I			experiment II		
	E_2 (pg ml^{-1})	huddling	pup grooming	E_2 (pg ml^{-1})	huddling	pup grooming
high E_2	26.8 ± 25.0	497 ± 22[b]	230 ± 59[b]	23.4 ± 16.3	300.7 ± 206	149 ± 118
low E_2	3.0 ± 4.5	100 ± 135	44 ± 72	5.9 ± 1.1	463.5 ± 128	259 ± 67

[a] All data reported as median ± semi-interquartile range.
[b] Significantly different from low E_2, $p < 0.05$.

treated with T + vehicle or T + FAD were not significantly different from males treated with E_2 + FAD or empty implants for huddling ($F_{1,48} = 0.53$, $p = 0.73$) or pup grooming behaviour ($F_{1,48} = 0.01$, $p = 0.94$). Males spent less time huddling ($F_{1,48} = 5.18$, $p = 0.03$) and pup grooming ($F_{1,48} = 6.88$, $p = 0.01$) in the first behaviour test (huddling: 236.6 ± 28.6 s, pup grooming: 122.0 15.8 s) compared to the second test (huddling: 289.9 ± 31.9 s, pup grooming: 153.4 ± 18.7 s). However, there was no test–treatment interaction for huddling ($F_{3,48} = 0.85$, $p = 0.47$) or pup grooming ($F_{3,48} = 1.2$, $p = 0.32$).

(c) *Hormone levels*

(i) *Testosterone levels*

In experiment I, T levels (1.21 ± 0.15 ng ml^{-1}) of males given T implants were not significantly correlated with huddling (Spearman's rank correlation, $r_S = 0.38$, $p > 0.05$) or pup grooming ($r_S = 0.17$, $p > 0.05$). Likewise DHT levels (0.69 ± 0.16 ng ml^{-1}) of males given DHT implants did not correlate with huddling ($r_S = 0.05$, $p > 0.05$) or pup grooming ($r_S = 0.05$, $p > 0.05$). In experiment II, T levels were not correlated with huddling within the T + vehicle ($r_S = 0.25$, $p > 0.05$), T + FAD ($r_S = -0.01$, $p = 0.05$) or empty ($r_S = 0.21$, $p > 0.05$) groups. Testosterone was also not correlated with pup grooming within the T + vehicle ($r_S = 0.29$, $p > 0.05$), T + FAD ($r_S = -0.01$, $p > 0.05$) or empty ($r_S = 0.19$, $p > 0.05$) groups. There were significant differences in T levels between these groups ($F_{2,36} = 13.45$, $p < 0.001$). Mean T levels in the T + vehicle (1.38 ± 0.28 ng ml^{-1}) and T + FAD (1.41 ± 0.27 ng ml^{-1}) groups were significantly greater than the empty (0.15 ± 0.04 ng ml^{-1}) group (LSD, $p < 0.001$), but were not significantly different from each other (LSD, $p = 0.63$).

(ii) *Oestradiol levels*

In experiment I, males with E_2 levels above the median showed significantly more huddling (table 1, $Z = 2.62$, $p < 0.01$) and pup grooming behaviour ($Z = 2.62$, $p < 0.01$) than males with E_2 levels below the median. However, in experiment II, males with E_2 levels above the median did not differ in huddling (table 1, $Z = -1.21$, $p = 0.23$) or pup grooming behaviour (table 1, $Z = -1.21$, $p = 0.23$) compared to males with E_2 levels below the median. There was no difference in E_2 levels across the two experiments ($Z = 0.13$, $p = 0.92$).

4. DISCUSSION

In both experiments, independent contrasts indicated that treatments in which E_2 was available (either directly or indirectly) resulted in higher levels of paternal behaviour compared to treatments in which E_2 was unavailable. Treatment with T or E_2 yielded significantly higher levels of paternal behaviour than treatment with DHT or empty implants, and aromatase inhibition blocked the positive effect of T on paternal behaviour, but not that of E_2. Taken together, these results demonstrate, for the first time to our knowledge, that E_2 promotes paternal behaviour in a paternal mammal. Thus, androgens only play an indirect role, acting either as a substrate for aromatase or possibly by promoting the expression of aromatase. These results may help to explain how paternal behaviour is expressed in several mammalian species when T levels are relatively high (Reburn & Wynne-Edwards 1999; Ziegler & Snowdon 2000).

(a) *Potential sites of aromatization and oestradiol action*

We propose that the brain is an important site of aromatization because brain areas associated with the control of parental behaviour have high levels of aromatase activity in male mammals. Aromatase activity in male mammals is high in the medial preoptic area (MPOA), medial amygdala (MA), and bed nucleus of the stria terminalis (BNST), whereas little or no aromatase activity is found in the lateral septum, hippocampus, or parietal cortex (rat, Roselli *et al.* (1985); rhesus monkey, Roselli *et al.* (1987); Syrian hamster, Hutchison *et al.* (1991)). The MPOA has been implicated as an important brain area regulating both maternal (Bridges 1996) and paternal behaviour (Kirkpatrick *et al.* 1994; Rosenblatt *et al.* 1996). The MA (Numan *et al.* 1993) and BNST (Fleming *et al.* 1980) have also been implicated in the control of maternal behaviour. In gonadectomized male rats treated with E_2 and progesterone implants, E_2 infused into the MPOA promotes parental behaviour, even though male rats normally do not show this behaviour (Rosenblatt & Ceus 1998). This suggests that E_2 regulation of paternal behaviour may be homologous to the effects of E_2 on maternal behaviour (Bridges 1996). However, there are important differences in the neuroendocrinology of paternal and maternal behaviour. Male mammals do not contend with the physiological demands of pregnancy and lactation, which have important effects on steroid and peptide

hormones (Tucker 1994). This difference may partially explain why E_2 does not promote maternal behaviour in reproductively experienced female rats (Rosenblatt 1990), but promotes paternal behaviour in reproductively experienced male California mice.

(b) *Peripherally circulating hormones*

Despite robust between-treatment group effects, there were no consistent relationships between T and paternal behaviours within treatment groups. These results suggest that there may be a threshold level of T (Hews & Moore 1997), above which consistently high levels of huddling and pup grooming are more likely to be expressed. A perplexing result is that in experiment I males with E_2 levels above the median showed higher levels of huddling and pup grooming than males with E_2 levels below the median, but this difference was absent in experiment II when FAD treatment was combined with E_2 implants. One possible explanation for this apparent interaction is that androgens produced by the adrenal glands (Bentvelsen *et al.* 1996) or brain (Zwain & Yen 1999) could have been increased by aromatase inhibition. FAD treatment could have increased non-gonadal androgens that would have otherwise been aromatized, thereby altering the relationship between E_2 levels and paternal behaviour. However, we believe these correlation data should be interpreted cautiously. Many of our hormonal measurements were at the limit of sensitivity for the assay and may have overlooked variability in individuals with lower E_2 levels. Regardless of how E_2 levels were correlated within treatment groups, E_2 treatment resulted in higher levels of paternal behaviour whether or not aromatase was inhibited.

(c) *Experience and neuroendocrine mechanisms of paternal behaviour*

There has been variability in the reported effects of T on male parental behaviour in other rodent species. One report found that a gonadectomy reduced parental behaviour in virgin male prairie voles (Wang & De Vries 1993), but a second report did not detect this effect (Lonstein & De Vries 1999). Clark & Galef (1999) used a slightly different design when they reported that castration increased paternal behaviour in Mongolian gerbils. They used males that were sexually inexperienced, but had cohabitated 10 days with a female that had been inseminated by a stud male. In all three of these reports, males lacked the normal sequence of stimuli normally experienced before having pups (reproductive experience). One hypothesis for the varying effects of a gonadectomy is that T and aromatase promote the maintenance, but not the onset of paternal behaviour. To our knowledge, it is unknown whether aromatase or steroid hormones promote the maintenance of paternal behaviour in reproductively experienced prairie voles or Mongolian gerbils. Changes in hormone levels before the birth of offspring probably play an important role in priming males to exhibit paternal behaviours. There is abundant evidence that male T and prolactin (PRL) levels, change after or during mating, pregnancy and parturition (common marmosets, Dixson & George (1982); cotton-top tamarins, Ziegler & Snowdon (2000); Djungarian hamsters, Reburn & Wynne-Edwards (1999); humans, Storey *et al.* (2000); Mongolian gerbils, Brown *et al.* (1995)). Although little is known about the function of PRL receptors in paternal mammals, male rats exposed to pups showed increased expression of PRL receptor mRNA in the cerebrum (Sakaguchi *et al.* 1996). PRL receptors have been located in the MPOA (Roky *et al.* 1996; Buntin 1996) and MA (Roky *et al.* 1996), and could therefore affect aromatase metabolism. Aromatization of T in the MPOA could also influence PRL levels via projections to the arcuate nucleus (Simerly & Swanson 1988), where dopaminergic neurons inhibit PRL secretion. Thus, changes in male T and PRL levels prior to parturition could have important effects on how brain areas associated with paternal behaviour process environmental stimuli associated with reproduction.

Environmental stimuli probably play an important role in the onset and maintenance of paternal behaviour. In the California mouse, chemosensory cues from a male's mate promote the maintenance of paternal behaviour in the absence of exposure to pups (Gubernick & Alberts 1989). In female rats, repeated exposure to pups promotes the onset of maternal behaviour (Orpen & Fleming 1987). In experiment II, averaged across all treatment groups, males showed higher levels of paternal behaviour when observed in a second behaviour test. This effect was not detected in experiment I, most probably because the intertest interval was one week between the first and second tests, whereas in experiment II the intertest interval was only one day. In contrast to Djungarian hamsters (Jones & Wynne-Edwards 2000) and California mice (Gubernick & Alberts 1987), Mongolian gerbil fathers avoid pups for 24 h following the birth of their first litter, but seek out pups during this period if they have prior exposure to pups (Clark & Galef 2000). Exposure to pups may be more important for stimulating the onset of paternal behaviour in Mongolian gerbils than in Djungarian hamsters or California mice.

5. CONCLUSIONS

These data show that T promotes paternal behaviour in male California mice through conversion to E_2, a step that probably takes place within the brain. As T levels are relatively high in several other mammalian species at times when paternal behaviour is expressed, this mechanism could be present in other species and warrants investigation. To date, most studies on male parental behaviour have focused on unmated males. Additional study of reproductively experienced males may prove helpful in determining whether T acts differently across species, or whether the effects of T on paternal behaviour depend on exposure to stimuli associated with reproductive experience. Male T, PRL and other hormone levels undergo changes after mating, pregnancy and parturition. These hormonal changes prior to the birth of offspring may lead to important changes within the brain that facilitate parental responses to the appropriate physiological and environmental stimuli. Future studies will investigate whether variation in aromatase activity within the brain is associated with variation in reproductive experience. Our work suggests that T may be an important physiological stimulus that contributes to the expression of mammalian paternal behaviour.

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