

Research report

Influence of gonadal hormones on the development of parental behavior in adult virgin prairie voles (*Microtus ochrogaster*)

Joseph S. Lonstein *, Geert J. De Vries

Center for Neuroendocrine Studies, Tobin Hall, Box 37720, University of Massachusetts, Amherst, MA 01003, USA

Received 24 September 1999; received in revised form 14 March 2000; accepted 14 March 2000

Abstract

Prairie voles (*Microtus ochrogaster*) are a socially monogamous species and both sexes are parental after the birth of pups. In contrast, sexually inexperienced adult prairie voles differ in their behavior towards pups such that virgin males are paternal whereas virgin females are often infanticidal. To test whether there exists a discrete perinatal 'sensitive period' during which gonadal hormones influence this behavior, and to distinguish between the relative contributions of estrogenic and androgenic mechanisms to this influence, prairie voles were exposed to testosterone propionate (TP), the anti-androgen flutamide, or the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) either prenatally via their pregnant dam for the last 15–19 days of the 22-day gestational period or postnatally on days 1–7. None of the treatments altered the high paternal responsiveness of males or the high infanticide rate in females when compared with controls. Females exposed prenatally to ATD, however, had levels of parental behavior that were significantly higher than the lowest levels observed in prenatally TP-treated females. These results suggest that sex differences in the parental behavior of adult virgin prairie voles are not generated exclusively by androgenic or estrogenic mechanisms during a restricted prenatal or early postnatal 'sensitive period' and that the parental behavior of virgin females may be more susceptible to any influence of gonadal hormones during development than males. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Estrogen; Infanticide; Maternal behavior; Monogamy; Paternal behavior; Perinatal; Sexual differentiation; Testosterone

1. Introduction

Prairie voles (*Microtus ochrogaster*) are a gregarious, cooperatively-breeding species that in natural environments often live in communal groups typically consisting of a pairbonded male and female and numerous offspring of varying ages from their successive series of litters [18,45]. Within both natural and laboratory settings, pairbonded males and females share in the rearing of their young [18,27,33,35,44,45]. When the details of their behavior towards pups are examined within the laboratory, the repertoire of nurturant behaviors displayed by males and females are strikingly similar. Indeed, both parents lick the pups and huddle with

them in distinctive 'nursing' postures, despite the fact that male prairie voles do not have developed nipples onto which pups can suckle and, therefore, receive different sensory inputs from the pups than do their mates [27,44]. In contrast, there is a large sex difference in the behavioral responses of sexually and parentally inexperienced adult prairie voles towards pups. Whereas adult virgin males that are reared after weaning with same-sex littermates are highly parental [2,3,28,54], their adult virgin female counterparts are often infanticidal [28,29]. These behavioral differences between virgin males and females do not depend on sex differences in adult levels of circulating gonadal hormones because neither gonadectomy during adulthood nor gonadectomy followed by identical estrogen treatments eliminate sex differences in their parental behavior [28].

* Corresponding author. Tel.: +1-413-5450526; fax: +1-413-5450996.

E-mail address: lonstein@cns.umass.edu (J.S. Lonstein).

Numerous sex differences in the brains and behavior of adult rodents can be influenced by differential exposure of males and females to testosterone during the perinatal period [17,59]. For example, testosterone which is normally secreted by fetal and neonatal rat testes can act upon neural androgen receptors, and upon estrogen receptors after aromatization to estradiol, to masculinize the behavior of the offspring. Female rat fetuses are protected from masculinization in part because their ovaries are relatively non-steroidogenic and maternal estrogen may be inactivated in the fetal bloodstream by being sequestered to α -feto-protein [17,59].

Similar to prairie voles, parental responsiveness in adult virgin rats is also sexually dimorphic, although female rats are more responsive towards pups than males. This sex difference in rat behavior is influenced by perinatal exposure to gonadal hormones (for review see Ref. [30]). To test whether perinatal exposure to gonadal hormones also influences the development of parental behavior in adult virgin prairie voles, we exposed voles to testosterone propionate (TP), the anti-androgen flutamide, or the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) either prenatally or postnatally. We hypothesized that either prenatal or postnatal testicular hormones acting through either an androgenic or estrogenic mechanism masculinize the parental behavior of male prairie voles, which in this species promotes responsiveness to pups. If these mechanisms are inhibited, males should display reduced responsiveness. Conversely, prenatal or postnatal treatment of females with TP may make their adult behavior more like males and promote maternal responses.

2. Materials and methods

2.1. Subjects

Subjects were male and female F2 and F3 generation prairie voles (*M. ochrogaster*) that were born and raised in our colony, which was established in 1996 at the University of Massachusetts, Amherst, from breeding stock originating from offspring of voles captured in 1994 from Urbana, IL, provided by Dr Betty McGuire (Smith College, Northampton, MA) and Dr Zuoxin Wang (Emory University, Atlanta, GA). Adult virgin female and male prairie voles were socially isolated for 3 days, after which the females were placed in the cage of an unfamiliar male. The animals were maintained on a 14:10 h light:dark cycle with an ambient temperature of 21°C. At all ages, the animals were housed in plastic cages (48 × 28 × 16 cm) containing wood chips, wood shavings, and substantial hay covering. Water and a food mixture containing cracked corn, whole oats, sunflower seeds, and Purina rabbit chow (ratio of

1:1:2:2) were available ad libitum. The subjects were weaned at 20 days of age and housed with their littermates in mixed-sex groups. At 30 days old the animals were rehoused in single-sex groups of 2–4 animals per cage. The subjects remained housed with their same-sex littermates until behavioral testing.

2.2. Procedure

To generate subjects that were treated prenatally, pregnant females received one of the following daily treatments beginning 1 week after pairing with a male prairie vole and continuing until the birth of the pups: (1) subcutaneous (SC) injection of 5 mg of the androgen receptor blocker flutamide (α,α,α -trifluoro-2-methyl-4-nitro-*m*-propionoluidide; Sigma, USA) dissolved in 200 μ l propylene glycol ($n = 8$ dams), (2) SC injection of 100 μ g testosterone propionate (TP) in 100 μ l sesame oil ($n = 18$ dams), or (3) SC injection of 1 mg of the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione; Steraloids, Wilton, NH) dissolved in 50 μ l sesame oil ($n = 8$ dams). We used flutamide to inhibit androgen receptor activity and ATD to reduce estrogenic activity because they have been demonstrated in similar or even smaller doses to prevent normal sexual differentiation of the central nervous system (e.g. Refs. [20,23,47,50,55]), external somatic morphology (e.g. Refs. [25,48]), and behavior (e.g. Refs. [12,42,49]) in other mammals. Due primarily to the incidence of abortion and stillbirth in many pregnant females treated daily with 100 μ g TP, as reported previously in this species [39], we were only able to produce and raise eight TP-treated litters. Two of these contained exclusively male pups which resulted in a final $n = 6$ in this group. Because preliminary data suggested that reduction of this dose to 70 μ g TP per day still did not produce a high percentage of live births, we continued to use 100 μ g/day to maintain consistency between our own and previously published reports [39]. Sesame oil was used as vehicle for the TP and ATD experiments because propylene glycol in the flutamide experiment appeared aversive to the pregnant dams after injection and was fatal to some pups when administered postnatally. Sesame oil never produced noticeable sickness in dams or pup fatality. Pregnant females that generated control subjects ($n = 20$ dams) received daily SC injection of propylene glycol or sesame oil vehicle. Within 24 h after birth, the pups from all groups were fostered to newly-parturient unmanipulated surrogate lactating prairie vole dams from our colony until weaning. To verify that the prenatal hormonal treatments were physiologically effective, anogenital distance was measured to the nearest mm for subjects from the prenatal studies when they were 30 days old.

Subjects that were treated postnatally received one of the following treatments beginning on the day of birth

and continuing for 7 days: (1) SC injection of 0.5 mg flutamide dissolved in 50 μ l propylene glycol ($n = 8$ litters), (2) SC injection of 1 mg TP in 50 μ l sesame oil ($n = 8$ litters), or (3) SC injection of 0.5 mg ATD in 50 μ l sesame oil ($n = 8$ litters). Control litters received daily SC injection of the appropriate volume of vehicle ($n = 24$ litters). All pups within a particular litter received the same treatment. All prenatal and all postnatal studies were performed concurrently over a period of 9 months.

2.3. Behavioral testing

One, and in a few cases two, animals of each sex per litter were used as subjects. At 90–95-days-old the subjects were removed from their home cage and placed in a clear Plexiglas cage of similar dimensions as their home cage that contained wood shavings, food and water, and a small amount of hay. After a 15-min habituation period, two 1–4 day-old prairie vole pups obtained from lactating prairie voles from our colony were scattered in the cage diagonally from where the subject was sitting. Although prairie vole dams typically give birth to three to five pups per litter, only two pups were presented during behavioral testing to keep the number of pups killed by infanticidal subjects at a minimum. Behavior of the subject was continuously recorded for 15 min with the aid of a custom-made data acquisition system designed to provide data on latency, frequency, and duration of numerous behaviors. Active behaviors directed toward pups included sniffing, licking, and carrying them from one place to another. Non-pup oriented activities recorded included self-grooming, exploration away from the pups, digging or nest construction, and eating or drinking. Measures of huddling behavior included actively hovering over the pups while performing other activities, and being quiescently positioned over the litter in one of three mutually-exclusive postures previously described in lactating rats: (1) kyphosis (upright crouch) characterized by all limbs placed on the ground in a splayed and rigid manner, depression of the head, and pronounced arching of the back that was strikingly similar to that seen in lactating rats [46]; both low and high-arched postures were included in this category; (2) hunched over the litter with body weight resting only on the hindlimbs and hindflanks, forelimbs elevated and drawn toward the body, and head passively resting on top of the litter [27]; and (3) laying prone on the litter mass with little or no limb support [31]. If a subject did not make contact with a pup within 3 min after the beginning of the test, the test was briefly paused while the pups were placed approximately 1 inch in front of the subject. In our experience, many virgin prairie voles do not quickly find and initiate contact with

pups in a relatively large testing arena, even though they are highly parental when the pups are offered directly to them. Total activity was the sum of the durations of all active behaviors recorded and the total time with the pups was calculated by the summed duration of actively hovering over the litter and quiescent nursing/huddling. Total quiescence was the sum of the duration of the time the subjects spent in all three quiescent postures. In cases where the subjects attacked a pup, the test was immediately terminated and the pup euthanized. In other cases, the pups were removed and returned to their parents after behavioral testing. A subject was considered parental if it spent ≥ 100 s in physical contact with the pups and was observed to lick them for ≥ 5 s. Retrieval of the pups was not included with these criteria because neither virgin nor postpartum prairie voles of either sex readily retrieve pups and often attend to them individually unless the scattered pups crawl to the parent [27,28].

2.4. Statistical analyses

Since no significant differences existed across the prenatal studies or across the postnatal studies in the percentage of control subjects of either sex displaying parental behavior, or in the details of the parental behavior of control males, the control groups were combined for comparison with experimental groups. The percentages of subjects in each group acting parentally were compared with the G -test of independence. This test often provides similar results as either the χ^2 or Fisher's exact probability tests but was better suited for our data because although the number of subjects in each comparison was fixed, the number that could potentially be parental was not [43]. It was not possible to compare females treated prenatally with TP with other groups using the G -test because none (0) of them acted parentally; Fisher's exact probability test which allows for comparisons with a group of zero responders was used in these two cases. Details of male subjects' parental behavior were analyzed with a one-way analysis of variance (ANOVA) that takes into account unequal sample sizes; the low percentage of parentally-responsive females precluded analysis of the details of their behavior. To reduce the influence of litter effects, anogenital distance was analyzed using the mean distance for males and females within each litter. A varying duration of time (12–72 h) after pairing is necessary before male-induced induction of estrus in female prairie voles and copulation takes place; the duration of prenatal treatments was, therefore, calculated retrospectively from the day of parturition. Differences were considered statistically significant if $P \leq 0.05$.

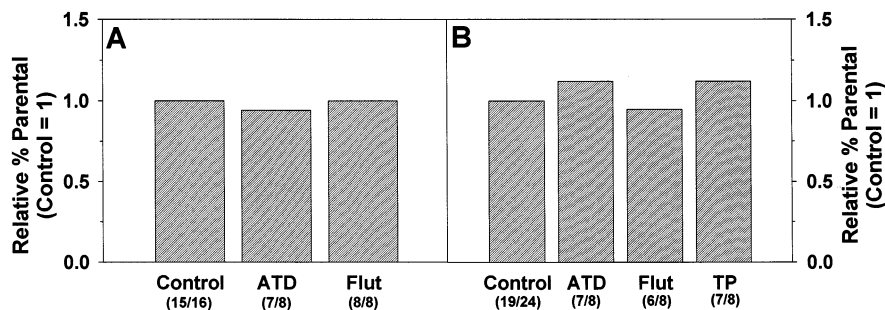


Fig. 1. Relative percentage of adult virgin male prairie voles acting parentally toward conspecific pups during 10 min tests after (A) prenatal exposure to vehicle ($n = 16$), ATD ($n = 8$), flutamide ($n = 8$), or (B) postnatal exposure to vehicle ($n = 24$), ATD ($n = 8$), flutamide ($n = 8$), or TP ($n = 8$). Controls from each study = 1.0. Numbers in parentheses are the number of parental subjects/total subjects in that group.

3. Results

3.1. Sex differences in parental behavior

The percentage of virgin adult subjects that spontaneously initiated contact with pups within 3 min after the beginning of testing, regardless of their subsequent behavior towards them, was similar for males (46%) and females (61%) ($G = 3.37$, d.f. = 1, $P \geq 0.05$). There were no significant differences within each sex according to treatment or the time of treatment in this percentage ($P_s \geq 0.1$). The behavior of control virgin males and females after contacting pups was strikingly different, however, such that 94% of prenatally-treated control males but only 24% of females were parental towards pups ($G = 18.96$, d.f. = 1, $P \leq 0.0001$). Similarly, in the postnatally treated subjects, 79% of control males were paternal, whereas only 42% of females acted maternally ($G = 7.28$, d.f. = 1, $P \leq 0.01$). The percentage of control males ($G = 1.77$, d.f. = 1, $P \geq 0.1$) or control females ($G = 1.49$, d.f. = 1, $P \geq 0.1$) that acted parentally did not significantly differ between the prenatal and postnatal experiments.

3.2. Effects of prenatal hormones on parental behavior of males

The number of days that fetuses were exposed to hormonal manipulations was 16.3 ± 0.6 (control), 18.5 ± 0.3 (flutamide), and 14.9 ± 0.2 (ATD) days. Although all pregnant females received their first injection beginning on the same day after pairing with a male, flutamide-exposed fetuses ended up being treated longer than other groups ($F(2,24) = 7.84$, $P \leq 0.003$).

Prenatal exposure to either the anti-androgen flutamide or to the aromatization inhibitor ATD had no significant effect on paternal behavior of adult males and all or most subjects from each group were highly parental during exposure to pups ($G_s \leq 0.3$, $P_s \geq 0.5$; Fig. 1A). Furthermore, no significant differences existed between groups in the details of their paternal behavior (Table 1).

Prenatal flutamide shortened (7.3 ± 0.3 mm), and prenatal ATD lengthened (11.3 ± 0.5 mm), anogenital distance in males when measured at 30 days old compared with controls (9.7 ± 0.2 mm) ($F(2,24) = 30.9$, $P \leq 0.0001$).

3.3. Effects of postnatal hormones on parental behavior of males

Similar to the prenatal studies, daily administration of TP, flutamide, or ATD for the first week after birth had no significant effects on responses towards pups with 75–88% of males in each group acting paternally ($G_s \leq 0.3$, $P_s \geq 0.5$; Fig. 1B). The details of their parental behaviors were quite similar, with the excep-

Table 1

Behavior (mean \pm S.E.M.) of parentally-responsive adult virgin male prairie voles during a 10-min test with pups after prenatal exposure to vehicle (control; $n = 16$), flutamide ($n = 8$), or ATD ($n = 6$)

Measure	Control	Flutamide	ATD	$F(2,27)$
<i>Latency (s)</i>				
Contact pups	153 \pm 29	131 \pm 38	157 \pm 32	0.13
<i>Hover over pups</i>				
From reunion	160 \pm 29	199 \pm 62	166 \pm 32	0.25
From first contact	8 \pm 1	9 \pm 3	14 \pm 3	3.33*
<i>Duration (s)</i>				
Hover over	327 \pm 35	288 \pm 63	320 \pm 34	0.20
Quiescence ^a	64 \pm 28	80 \pm 30	38 \pm 23	0.32
Carry pups	0.4 \pm 0.2	1.5 \pm 1.2	0.3 \pm 0.1	1.02
Lick pups	181 \pm 23	192 \pm 38	188 \pm 25	0.05
Nest/burrow	3 \pm 2	5 \pm 3	9 \pm 9	0.49
Explore	48 \pm 19	25 \pm 7	67 \pm 14	0.89
Self-groom	39 \pm 12	50 \pm 21	60 \pm 12	0.47
Total activity ^b	307 \pm 36	294 \pm 29	360 \pm 28	0.60
TTWP ^c	390 \pm 33	368 \pm 80	358 \pm 33	0.11

^a Includes kyphosis, hunched, and prone postures.

^b Includes eating and drinking which were of short duration and not displayed by all subjects.

^c TTWP, total time with pups (hovering over + quiescence).

* $P \leq 0.06$.

The generally low levels of responding in females precluded statistical analyses.

Table 2
Behavior (mean \pm S.E.M.) of parentally-responsive virgin adult male prairie voles during a 10-min test with pups after postnatal administration of either vehicle (control; $n = 19$), the anti-androgen flutamide ($n = 6$), ATD ($n = 8$), or TP ($n = 7$)

Measure	Control	Flutamide	ATD	TP	$F(3,36)$
<i>Latency (s)</i>					
Contact pups	143 \pm 32	183 \pm 50	106 \pm 32	180 \pm 41	0.62
<i>Hover over pups</i>					
From reunion	148 \pm 32	187 \pm 51	116 \pm 31	194 \pm 44	0.64
From first contact	5 \pm 1	4 \pm 3	10 \pm 1	14 \pm 8	1.99
<i>Duration (s)</i>					
Hover over	326 \pm 34	367 \pm 39	296 \pm 17	272 \pm 55	0.70
Quiescence ^a	49 \pm 11a	31 \pm 14a	104 \pm 42b	3 \pm 3a	3.32*
Carry pups	1.8 \pm 1.2	0.8 \pm 0.7	4.4 \pm 2.5	0.3 \pm 0.1	1.12
Lick pups	184 \pm 22	181 \pm 23	179 \pm 20	132 \pm 20	0.79
Nest/Burrow	7 \pm 3	8 \pm 5	0 \pm 0	19 \pm 10	1.86
Explore	75 \pm 18	41 \pm 11	82 \pm 28	130 \pm 28	1.69
Self-groom	34 \pm 10a	73 \pm 22ab	35 \pm 11a	89 \pm 24b	3.03*
Total activity ^b	334 \pm 30	329 \pm 28	343 \pm 40	416 \pm 19	1.07
TTWP ^c	376 \pm 34	399 \pm 47	400 \pm 29	276 \pm 54	1.39

^a Includes kyphosis, hunched, and prone postures.

^b Includes eating and drinking which were of short duration and not displayed by all subjects.

^c TTWP, total time with pups (hovering over + quiescence).

* ANOVA $P \leq 0.05$. Significant post-hoc differences between groups indicated by different letters, $P \leq 0.05$.

The generally low levels of responding in females precluded statistical analyses.

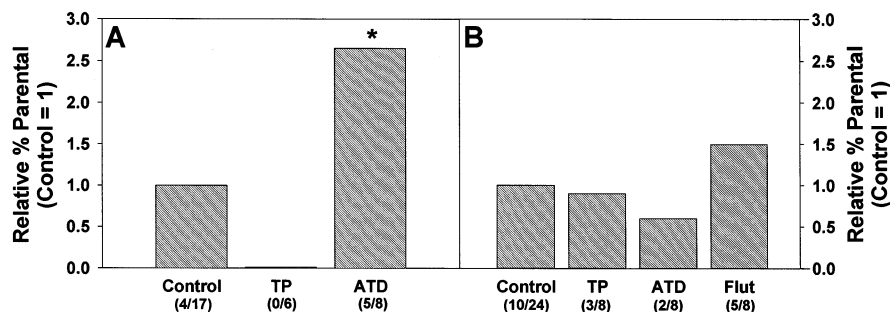


Fig. 2. Relative percentage of adult virgin female prairie voles acting parentally toward conspecific pups during 10 min tests after (A) prenatal exposure to vehicle ($n = 17$), TP ($n = 6$), ATD ($n = 8$) or (B) postnatal exposure to vehicle ($n = 24$), TP ($n = 8$), ATD ($n = 8$), or flutamide ($n = 8$). Controls from each study = 1.0. Numbers in parentheses are the number of parental subjects/total subjects in that group. *Fisher's exact probability test, $P \leq 0.05$ compared with TP-treated females.

tion of ATD-treated males spending more time quiescently postured over pups than the other groups and TP-treated males self-grooming more than control or flutamide-treated males (Table 2).

3.4. Effects of prenatal hormones on parental behavior of females

The number of days that females were prenatally exposed to hormonal manipulations did not differ between groups: 14.9 \pm 0.2 days (control), 15.0 \pm 0.4 days (TP), 14.8 \pm 0.5 days (ATD) ($F(2,23) = 0.02$, $P \geq 0.9$).

Most control females (77%) attacked pups, as did all females exposed prenatally to TP (Fisher's exact proba-

bility test, $P \geq 0.5$). In contrast, many females (63%) exposed prenatally to ATD were parental, though differences in this percentage compared with controls only approached statistical significance ($G = 3.5$, d.f. = 1, $P \leq 0.065$). The difference between females exposed prenatally to TP and ATD in the percentage acting parentally, however, was significant (Fisher's exact probability test, $P \leq 0.04$; Fig. 2A). The small percentage of maternally-acting females sniffed, licked, and huddled over the pups in a manner similar to that of males.

Prenatal exposure to TP significantly lengthened anogenital distance in female prairie voles compared with controls: 4.5 \pm 0.2 mm (control), 5.7 \pm 0.3 mm (TP), 5.1 \pm 0.4 mm (ATD) ($F(2,23) = 4.73$, $P \leq 0.02$).

3.5. Effects of postnatal hormones on parental behavior of females

Postnatal administration of TP, ATD, or flutamide had no effect on the percentage of females acting parentally during exposure to pups, with relatively few females in each group acting maternally ($G_s \leq 0.74$, all $P_s \geq 0.3$; Fig. 2B). There were no obvious differences between the parental behavior displayed by the small percentage of maternally-acting females within each group and that displayed by males.

4. Discussion

The results of the present studies suggest that inhibition of either androgenic or estrogenic activity during a discrete period before or after birth does not significantly influence the development of a sex difference in the parental behavior of adult virgin prairie voles. Males under all circumstances were highly parental, whereas a large percentage of females in all groups attacked the pups. The small percentage of females that did not kill the pups acted parentally in a manner quite similar to males, as demonstrated previously [28]. There is apparently no distinct prenatal or postnatal sensitive period during which estrogens or androgens alone can generate this sex difference in adult behavior. This suggests that gonadal hormones contribute to the development of parental behavior in virgin prairie voles differently than in other rodents, including laboratory rats.

4.1. Perinatal hormones and parental behavior

It appears that prenatal exposure to gonadal hormones is not necessary for later paternal behavior in male prairie voles since inhibition of gonadal hormone activity had no effect on their adult interactions with pups. Furthermore, the normal parental behavior in a small number of males that were prenatally exposed to TP indicates that prenatal androgen also does not influence their later parenting (Lonstein and De Vries, unpublished observations). The effects of inhibiting prenatal gonadal hormone activity on the parental responsiveness of adult male rats have not been examined, so it is unknown whether these hormones influence parental responding in this species.

Postnatal gonadal hormones influence the development of sex differences in parental behavior differently in voles than they do in rats. Castration of male rats prior to puberty feminizes parental behavior by decreasing infanticide and increasing parental responsiveness, with removal of the testes soon after birth being most effective in feminizing their responses towards pups [32,37,40,41]. This does not appear to be the case

in male prairie voles because the inhibition of androgenic or estrogenic activity during the first week of life had little effect on their responses towards pups during adulthood. Furthermore, neonatal castration only reduces, but does not completely feminize, later parental responding in male prairie voles (Lonstein, Rood, and De Vries, unpublished data). Our finding that postnatal TP had only minor effects on the behavior of males is inconsistent with a previous report [39] showing that similar treatment significantly decreased the amount of time that they spent with pups. This difference may be explained by the fact that, whereas we investigated the behavior of adult males, Roberts et al. [39] examined juvenile and pubertal males. We have no explanation for the increases in quiescence over pups displayed by males treated postnatally with ATD or in self-grooming by TP-treated males, but these increases may be related to organizational effects of androgens or estrogens on patterns of locomotor activity [58].

The present results do not necessarily exclude a role for gonadal hormones in the development of parental behavior in virgin prairie voles. Gonadal hormones may influence the development of parental behavior via androgenic and estrogenic mechanisms simultaneously, prenatally as well as neonatally. Additionally, 'critical periods' for sexual differentiation may extend well beyond the perinatal period [9,13]. In other mammals, however, manipulation of gonadal hormones either prenatally or postnatally is sufficient to change the development of some sexually dimorphic behaviors (e.g. Refs. [5,7,14,42,49,56]), including parental behavior in virgin rats (see Ref. [30]). Manipulations of hormone activity during both the prenatal and postnatal periods can be more effective in some cases (e.g. Refs. [6,23,47,56]). This does not appear to be the case in prairie voles because many males that are sheltered from the actions of gonadal hormone throughout most of their life after prenatal administration of both flutamide and ATD followed by neonatal castration are still paternal during adulthood (Lonstein, Rood, and De Vries, unpublished data).

It is also interesting to consider that the hormonal dependency of parental behavior in male prairie voles was altered by the pre- or postnatal manipulations in a manner that could not be detected in the present experiments. We previously demonstrated that in addition to the large sex difference in responsiveness toward pups, the hormonal dependence of this behavior differs between the sexes because castration at weaning or during adulthood does not affect the behavior of either sex toward pups ([28,29], Lonstein, unpublished observations), but chronic estradiol treatment tends to promote maternal behavior in females [28]. Therefore, gonadal hormones under certain conditions can stimulate parental behavior in females. If prenatal or postnatal blockade of androgenic or estrogenic activity feminized

the parental responsiveness of males in terms of making it more dependent on hormonal stimulation, the presence of endogenous testosterone may have stimulated their parental behavior, masking the effects of the perinatal treatment. In such a case, castration during adulthood may have reduced parental behavior in these animals.

In contrast to males, the present results indicate that prenatal gonadal hormones can influence the development of parental behavior in female prairie voles. Female prairie voles resemble female rats in this respect because prenatal testosterone masculinizes parental responsiveness in female rats, whereas neonatal testosterone treatment has little or no effect [11,24,30]. However, we expected prenatal TP treatment to masculinize females' parental behavior, but if anything it seemed to promote infanticide. The apparently opposite effects of prenatal TP and ATD on their behavior towards pups suggests that any effects of TP on parental responsiveness depend on the aromatization of testosterone to estradiol. This does not necessarily mean that inhibiting aromatization of fetal testosterone produced these effects since maternal production of estrogens was also inhibited. Endogenous hormones from adjacent male littermates [53], steroidogenic activity of the female fetus' own ovaries or adrenal glands, or even ovarian secretions from their mother [57] may also normally influence the development of parental behavior in females. If this is the case it is unclear why these hormones would not also inhibit parental behavior in males. Since neither TP or ATD produced a significant change in the percentage of females acting parentally when compared to controls, but only when compared to each other, these mechanisms may have only relatively small influences on the development of parental responsiveness in females. Perhaps more pronounced behavioral effects could have been obtained with larger sample sizes or by increasing the already high doses of the agents used to alter gonadal hormone activity. Furthermore, although the effects of perinatal hormones on parental responding in rats can be affected by the novelty of the testing condition [11], most unmanipulated non-lactating female prairie voles are infanticidal whether they are tested in their home cage [52] or in a novel cage ([28,29], present report).

Although not statistically significant, there was a noticeable reduction between the prenatal and postnatal studies in the magnitude of the sex difference in parental behavior. Pregnant rats exposed to stress produce offspring with reduced sex differences in parental responsiveness [26], possibly resulting from increased maternal or fetal adrenal androgen or glucocorticoid secretion. Postnatal handling and injection for the first week after life may produce similar effects in prairie voles. The explanation for this behavioral shift in the control groups of voles may not be that simple, though,

because direct administration of corticosterone to neonatal female prairie voles reduces later parental behavior, not increases it [39].

The function, if any, of the sex differences in parental behavior of virgin prairie voles is unknown because it may not be particularly common in natural environments. Female prairie voles in our and numerous other laboratories are removed from the natal nest at weaning and housed with female littermates. This social environment may promote infanticide not because of the absence of male littermates [29], especially considering that even complete social isolation after weaning has no effect on their aggressive behavior [21,29], but because of the absence of their sire and dam. We have recently demonstrated that female prairie voles that remain in the presence of their parents after weaning are highly parental, even if the dam does not give birth to subsequent litters of pups [29]. In contrast to some laboratory-reared females, many females in natural environments do not disperse from the natal nest but rather continue to live with their parents and younger siblings. Furthermore, juvenile virgin females may gain parental experience by interacting with younger siblings if their dams are impregnated during a postpartum estrus and give birth to another litter. This early experience with younger pups permanently renders virgin females more parental [29]. Only in cases where females disperse from the natal nest without prior early experience with pups would they behave differently from males and have a propensity for infanticide.

4.2. *Prairie voles: a unique model for sexual differentiation*

Our present experiments add to a growing list of studies suggesting that gonadal hormones do not play a traditional role in the development of sexually dimorphic behavior in voles. Unlike neonatal castration of rats, which demasculinizes sexual [34] and aggressive [8] behaviors, castration has little or no effect on these behaviors in gray-tailed (*M. canicaudus*) or prairie voles [15,36,38]. Also dissimilar to rats [10,16,19,22,51], prenatal inhibition of androgenic or estrogenic activity in male voles and TP treatment of female voles have little or no effects on their sexual behavior [36,38]. The effects of postnatal TP treatment on sexual behavior in prairie voles are even more unusual because not only does it defeminize sexual behavior in females, it also demasculinizes it in males [38]. Our results indicate that the unusual effects of gonadal hormones on the development of sexually dimorphic behaviors in prairie voles also extend to their parental behavior.

Whereas the actions of perinatal gonadal hormones on sexual differentiation in prairie voles differs from those actions in rats, they are reminiscent of the actions of these hormones in birds. For example, the song

system in zebra finches is sexually dimorphic, but while females are susceptible to the masculinizing effects of perinatal estrogens, hormonal manipulations have no apparent effect on developing males [1]. The effects of perinatal gonadal hormones on the adult behavior of other mammals including ferrets, guinea pigs, and primates [4] offer additional examples of species in which hormones regulate the development of sexually dimorphic behaviors in ways that are unlike that in laboratory rats. These findings underscore the importance of considering that different mechanisms may be responsible for sexual differentiation in different animal species.

Acknowledgements

This research was supported by NIMH grant MH47538 to G.J. De Vries and NICHD postdoctoral NRSA HD08392 to J.S. Lonstein. The authors thank Ross Lonstein for creating the data acquisition software used in the present studies and Katie Loeser and Ben Rood for assistance with behavioral observations.

References

- [1] Arnold AP. Experimental analysis of sexual differentiation of the zebra finch brain. *Brain Res Bull* 1997;44:503–7.
- [2] Bamshad M, Novak MA, De Vries GJ. Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster* and meadow voles. *J Neuroendocrinol* 1993;5:247–55.
- [3] Bamshad M, Novak MA, De Vries GJ. Cohabitation alters vasopressin innervation and paternal behaviors in prairie voles (*Microtus ochrogaster*). *Physiol Behav* 1994;56:751–8.
- [4] Baum MJ. Differentiation of coital behavior in mammals: a comparative analysis. *Neurosci Biobehav Rev* 1979;3:265–84.
- [5] Baum MJ, Tobet SA. Effect of prenatal exposure to aromatase inhibitor, testosterone, or antiandrogen on the development of feminine sexual behavior in ferrets of both sexes. *Physiol Behav* 1986;37:111–8.
- [6] Baum MJ, Carrol RS, Cherry JA, Tobet SA. Steroidal control of behavioural, neuroendocrine and brain sexual differentiation: studies in a carnivore, the ferret. *J Neuroendocrinol* 1990;2:401–18.
- [7] Baum MJ, Erskine MS, Kornberg E, Weaver CE. Prenatal and neonatal testosterone exposure interact to affect differentiation of sexual behavior and partner preferences in female ferrets. *Behav Neurosci* 1990;104:183–98.
- [8] Bergvall AH, Hansen S. Female-enhanced aggression in male rats: effects of genital anesthesia, castration, or preoptic lesions. *Behav Neurosci* 1990;104:348–55.
- [9] Bloch GJ, Mills R, Gale S. Prepubertal testosterone treatment of female rats: defeminization of behavior and endocrine function in adulthood. *Neurosci Biobehav Rev* 1995;19:177–86.
- [10] Brand T, Kroonen J, Mos J, Slob AK. Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations. *Horm Behav* 1991;25:323–41.
- [11] Bridges RS, Zarrow MX, Denenberg VH. The role of neonatal androgen in the expression of hormonally induced maternal responsiveness in the adult rat. *Horm Behav* 1973;4:315–23.
- [12] Clemens LG, Gladue BA, Coniglio LP. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Horm Behav* 1978;10:40–53.
- [13] Davis EC, Shryne JE, Gorski RA. A revised critical period for the sexual differentiation of the sexually dimorphic nucleus of the preoptic area in the rat. *Neuroendocrinology* 1995;62:579–85.
- [14] Davis PG, Chaptal CV, McEwen BS. Independence of the differentiation of masculine and feminine sexual behavior in rats. *Horm Behav* 1979;12:12–9.
- [15] Demas GE, Moffatt CA, Drazen DL, Nelson RJ. Castration does not inhibit aggressive behavior in adult male prairie voles. *Physiol Behav* 1999;66:59–62.
- [16] Dunlop JL, Gerall AA, Carlton SF. Evaluation of prenatal androgen and ovarian secretions on receptivity in female and male rats. *J Comp Physiol Psychol* 1978;92:280–8.
- [17] Forger NG. The development of sex differences in the nervous system. In: Blass, E.M. editor. *Handbook of Behavioral Neurobiology*, Vol. 12. 1999 (in press).
- [18] Getz LL, McGuire B. Communal nesting in prairie voles (*Microtus ochrogaster*): formation, composition, and persistence of communal groups. *Can J Zool* 1997;75:525–34.
- [19] Gladue BA, Clemens LG. Androgenic influences on feminine sexual behavior in male and female rats: defeminization blocked by prenatal antiandrogen treatment. *Endocrinology* 1978;103:1702–9.
- [20] Grisham W, Casto JM, Kashon ML, Ward IL, Ward OB. Prenatal flutamide alters sexually dimorphic nuclei in the spinal cord of male rats. *Brain Res* 1992;578:69–74.
- [21] Harper SJ, Batzli GO. Are staged dyadic encounters useful for studying aggressive behaviour of arvicoline rodents? *Can J Zool* 1997;75:1051–8.
- [22] Hoepfner BA, Ward IL. Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. *Behav Neurosci* 1988;102:61–5.
- [23] Houtsmuller EJ, Brand T, de Jonge FH, Joosten RNJMA, van de Poll NE, Slob AK. SDN-POA volume, sexual behavior, and partner preference of male rats affected by perinatal treatment with ATD. *Physiol Behav* 1994;56:535–41.
- [24] Ichikawa S, Fujii Y. Effect of prenatal androgen treatment on maternal behavior in the female rat. *Horm Behav* 1982;16:224–33.
- [25] Imperato-McGinley R, Sanchez RS, Spencer JR, Yee B, Vaughan ED. Comparison of the effects of the 5 α -reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose–response studies. *Endocrinology* 1991;131:1149–56.
- [26] Kinsley C, Bridges RS. Prenatal stress and maternal behavior in intact and virgin rats: response latencies are decreased in males and increased in females. *Horm Behav* 1988;22:76–89.
- [27] Lonstein JS, De Vries GJ. Comparison of the parental behavior of pairbonded female and male prairie voles (*Microtus ochrogaster*). *Physiol Behav* 1999;66:33–40.
- [28] Lonstein JS, De Vries GJ. Sex differences in the parental behavior of adult virgin prairie voles: independence from gonadal hormones and vasopressin. *J Neuroendocrinol* 1999;11:441–9.
- [29] Lonstein JS, De Vries GJ. Social influences on maternal behavior in adult virgin female prairie voles (*Microtus ochrogaster*). *J Comp Psychol* 2000 (submitted).
- [30] Lonstein JS, De Vries GJ. Sex differences in parental behavior. *Neurosci Biobehav Rev* 2000 (submitted).
- [31] Lonstein JS, Stern JM. Role of the midbrain periaqueductal gray in maternal nurturance and aggression: *c-fos* and electrolytic lesion studies in lactating rats. *J Neurosci* 1997;17:3364–78.
- [32] McCullough J, Quadagno DM, Goldman BD. Neonatal gonadal hormones: effect on maternal and sexual behavior in the male rat. *Physiol Behav* 1974;12:183–8.

- [33] McGuire B, Novak MA. A comparison of maternal behaviour in the meadow vole (*Microtus pennsylvanicus*), prairie vole (*M. ochrogaster*) and pine vole (*M. pinetorum*). *Anim Behav* 1984;32:1132–41.
- [34] Meisel RL, Sachs BD. The physiology of male sexual behavior. In: Knobil E, Neill JD, editors. *The Physiology of Reproduction*, vol. 2. New York: Raven Press, 1994:3–106.
- [35] Oliveras D, Novak MA. A comparison of paternal behaviour in the meadow vole *Microtus pennsylvanicus*, the pine vole *M. pinetorum* and the prairie vole *M. ochrogaster*. *Anim Behav* 1986;34:519–26.
- [36] Petersen SL. Perinatal androgen manipulations do not affect feminine behavioral potentials in voles. *Physiol Behav* 1986;36:527–31.
- [37] Quadagno DM, Rockwell J. The effect of gonadal hormones in infancy on maternal behavior in the adult rat. *Horm Behav* 1972;3:55–62.
- [38] Roberts RL, Zullo AS, Carter CS. Sexual differentiation in prairie voles: the effects of corticosterone and testosterone. *Physiol Behav* 1997;62:1379–83.
- [39] Roberts RL, Zullo A, Gustafson EA, Carter CS. Perinatal steroid treatments alter alloparental and affiliative behavior in prairie voles. *Horm Behav* 1996;30:576–82.
- [40] Rosenberg KM, Denenberg VH, Zarrow MX, Frank BL. Effects of neonatal castration and testosterone on the rat's pup-killing behavior and activity. *Physiol Behav* 1971;7:363–8.
- [41] Rosenberg PA, Herrenkohl LR. Maternal behavior in male rats: critical times for the suppressive action of androgens. *Physiol Behav* 1976;16:293–7.
- [42] Roy MM. Effects of prenatal testosterone and ATD on reproductive behavior in guinea pigs. *Physiol Behav* 1991;51:105–9.
- [43] Sokol RR, Rohlf FJ. *Biometry*, 2nd edn. Freeman Press, N.Y. 1981.
- [44] Solomon NG. Comparison of parental behavior in male and female prairie voles (*Microtus ochrogaster*). *Can J Zool* 1993;71:434–7.
- [45] Solomon NG, Getz LL. Examination of alternative hypotheses for cooperative breeding in rodents. In: Solomon NG, French JA, editors. *Cooperative Breeding in Mammals*. Cambridge: Cambridge University Press, 1997:199–230.
- [46] Stern JM. Somatosensation and maternal care in Norway rats. In: Slater PJ, Rosenblatt JS, Snowden CT, editors. *Parental Care: Evolution, Mechanisms, and Adaptive Significance*, *Advances in the Study of Behavior*, vol. 25. New York: Academic Press, 1996:243–94.
- [47] Swaab DF, Slob AK, Houtsmuller EJ, Brand T, Zhou JN. Increased number of vasopressin neurons in the suprachiasmatic nucleus (SCN) of 'bisexual' adult male rats following perinatal treatment with the aromatase blocker ATD. *Dev Brain Research* 1995;85:273–9.
- [48] Tate-Ostroff BA, Bridges RS. Nipple development and pup-induced prolactin release in male rats treated prenatally with the antiandrogen flutamide. *Psychoneuroendocrinology* 1988;13:309–16.
- [49] Thornton JE, Irving S, Goy RW. Effects of prenatal antiandrogen treatment on masculinization and defeminization of guinea pigs. *Physiol Behav* 1991;50:471–5.
- [50] Tobet SA, Zahniser DJ, Baum MJ. Differentiation in male ferrets of a sexually dimorphic nucleus of the preoptic/anterior hypothalamic area requires prenatal estrogen. *Neuroendocrinology* 1986;44:299–308.
- [51] Vega-Matuszczyk JV, Larsson K. Sexual preference and feminine and masculine sexual behavior of male rats prenatally exposed to antiandrogen or antiestrogen. *Horm Behav* 1995;29:191–206.
- [52] Villalba C, Boyle PA, De Vries GJ. Effects of serotonin reuptake inhibitor fluoxetine on social behaviors in male and female prairie voles. *Soc Neurosci Abst* 1997;23:1087.
- [53] Vom Saal FS. Sexual differentiation in litter bearing mammals: influence of sex of adjacent fetuses in utero. *J Anim Sci* 1989;67:1824–80.
- [54] Wang Z, De Vries GJ. Testosterone effects on paternal responsiveness and vasopressin immunoreactive projections in prairie voles (*Microtus ochrogaster*). *Brain Res* 1993;631:156–60.
- [55] Weaver CE, Baum MJ. Differential regulation of aromatase by androgen in adult and fetal ferrets. *Endocrinology* 1991;128:1247–54.
- [56] Whalen RE, Gladue BA, Olsen KL. Lordotic behavior in male rats: genetic and hormonal regulation of sexual differentiation. *Horm Behav* 1986;20:73–82.
- [57] Witcher JA, Clemens LG. A prenatal source for defeminization of female rats is the maternal ovary. *Horm Behav* 1987;21:36–43.
- [58] Wollnik F, Dohler KD. Effects of adult or perinatal hormonal environment on ultradian rhythms in locomotor activity of laboratory LEW/Ztm rats. *Physiol Behav* 1986;38:229–240.
- [59] Yahr P. Sexual differentiation of behavior in the context of developmental psychobiology. In: Blass EM, editor. *Handbook on Behavioral Neurobiology*, vol. 9. New York: Plenum, 1988:197–243.