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Stability and Potential Inheritance of Infanticidal Behavior in Prairie Voles

ABSTRACT: *Naïve female prairie voles show significant variability in their behavioral response to newborns. We investigated whether that behavioral response (a) was related to the quality of postpartum maternal behavior; (b) was affected by postpartum maternal experience; and (c) could be selectively bred. The behavior of females was recorded in three conditions: as naïve in a nonreproductive context, as single lactating (no male present), and as experienced mother in a nonreproductive context. Finally, females and males with similar behavioral response to newborns were selectively bred for three generation. Males were removed before the offspring was born. Our results revealed that (a) naïve females that attacked pups, spent more time distant from them after parturition than those that were maternal or ignored the pups ($p < .05$); (b) postpartum maternal experience did not reverse infanticidal behavior; and (c) at the third generation of selective breeding, 90% of the offspring of females that were nonmaternal as virgins, behaved as their mothers. These findings suggest that the infanticidal behavioral response is a stable behavioral trait and might be passed to the offspring. © 2010 Wiley Periodicals, Inc.* Dev Psychobiol 52: 825–832, 2010.

Keywords: *experience; infanticidal; maternal; Microtus ochrogaster; selective breeding*

INTRODUCTION

Parental behavior in female and male prairie voles, as in other rodent species, can be induced by cohabitation with pups in a nonreproductive context. Both juveniles and adults of many species will display parental behavior after continuous exposure to newborns (Bridges, Zarrow, Goldman, & Denenberg, 1974; Brunelli & Hofer, 1990; Mayer, 1983; Rosenblatt, 1967). This type of parental response in a nonreproductive context is significantly influenced by the socio-emotional status of the individual (Clinton et al., 2007; Olazabal & Young, 2005). For example, most (∼60%) prairie voles display parental behavior in less than 2 min. of being exposed to newborns (Olazabal & Young, 2005; Roberts, Williams, Wang, & Carter, 1998). However, significant intraspecific variability in the behavioral response to pups has been reported within and among laboratories (Lonstein & DeVries, 2001; Olazabal & Young, 2005).

Spontaneous parental or infanticidal behavior are likely triggered by a combination of complex multisensory stimuli and a particular social, emotional, and physiological status of the subject that contribute to process pup related stimuli as attractive or aversive (Febo et al., 2008; Mayer, 1983; Numan & Insel, 2003). Postpartum maternal care also varies significantly within a species, including differences in the quality of nursing or crouching over pups, and the time and frequency of licking and grooming (Francis, Champagne, & Meaney, 2000; Stern & Johnson, 1990). In the first experiment, we investigated whether there was a relationship between the behavioral response toward pups displayed by naïve female prairie voles, and the quality of maternal behavior displayed by them during the first 4 days after parturition.

Unpublished observations in our laboratory revealed that female prairie voles are very consistent in their

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behavioral response toward pups (maternal, or nonmaternal), at least when tested in three or four consecutive days. However, whether the experience of being lactating, having their own offspring, and taking care of them for 20 days facilitate spontaneous maternal behavior was unknown. Therefore we considered interesting to test maternal behavior 1 month after females weaned their own pups. Previous studies in rats, among other species, show that maternal experience increases the incidence of pup-induced maternal behavior (Bridges, 1975, 1977; Cohen & Bridges, 1981; Orpen & Fleming, 1987). Therefore we tested each female prairie voles for their response to pups in three conditions, first as inexperienced virgin tested in a nonreproductive context (naïve females exposed to pups), second as primiparous tested for 4 days after parturition (lactating maternal behavior), and third as experienced tested again in a nonreproductive context (experienced females exposed to pups). We expected that if lactating maternal experience positively modified the behavioral response toward pups, most females that attacked pups should then display spontaneous maternal behavior in the third experienced testing condition.

Finally, we investigated whether the behavioral response (maternal or nonmaternal) displayed by female prairie voles when exposed to newborns for the first time could be selectively bred. Previous selective breeding studies have successfully bred line of animals for different levels of anxiety, response to novelty, and aggressiveness, among other behaviors (Brunelli, Vinocur, Soo-Hoo, & Hofer, 1997; Parmigiani, Palanza, Rodgers, & Ferrari, 1999; Stead et al., 2006; Touma et al., 2008). If females show consistency in their behavioral response toward pups in a nonreproductive context, and the differences among these females are also present after parturition, then it is possible that spontaneous or infanticidal behaviors are stable behavioral traits, can be inherited by the offspring, and passed to next generations by genetic, or epigenetic mechanisms.

METHODS

Subjects

All animals were from the colony maintained at the Yerkes Laboratory Animal Facility at Emory University. This facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Prairie voles in the colony were derived from Illinois, USA field caught stock. Animals from the field were most recently introduced into the colony in 1995. All animals were weaned at 21 days of age, maintained in same-sex (in general siblings) groups of 2–3 in cages 28 cm × 17 cm × 13 cm with transparent Plexiglas Walls under a 12/12-hr dark/light cycle and a stable environmental temperature of 22°C with access to food (LabDiet® rabbit) and water ad libitum. Bed-ócobs® Laboratory Animal Bedding (Maumee, OH) was used as bedding material. Cages in this animal colony were regularly changed once a week.

All procedures used in this study followed the ethical guidelines of the American Psychological Association and the standards approved by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council). Additional adult lactating females not included in the experiment served as donors of pups for the nonlactating maternal behavior tests.

Screening of Maternal and Nonmaternal Naïve Females in a Nonreproductive Context

Females were individually housed in a clean cage and allowed to habituate for 45–90 min before the maternal behavior test began. The same experimenter scored all behaviors.

Two pups (2–5 days old) were placed into the cage opposite to where the subject was located at the time the lid was opened. The following behaviors were scored for 15 min from an observer placed about 1 m away from the cage: number of animals that attack, or display maternal behavior. Our criteria for considering an animal ''spontaneously maternal'' has been described before (Olazabal & Young, 2005) and includes all those animals that lick and gather the pups (retrieving, pushing, or handling them, and/or moving herself where they are), and spend >30 s adopting crouching postures over them. Generally, animals that crouch over the pups have already licked and gathered them and will spend most of the testing time adopting crouching posture. Animals that neither reached the criteria for maternal behavior nor attacked the pups during the 15 min test period were categorized as females that 'neglected or ignored' the pups. Pups were removed from their cage at the end of the test, or immediately after suffered an attack by the subject. Subjects that attacked were categorized as infanticidal females. Pups with serious injuries were euthanized immediately.

Postpartum Maternal Behavior Test (Mother Disturbed)

After the first maternal test, all females were paired with males to induce pregnancy. A group of seven females that attacked pups, four that ignored them, and six that displayed spontaneous maternal behavior were studied in detail during the postpartum period. Males were removed from the pregnant female cage after 2 weeks of pairing. Around 24–27 days after pairing males and females, these 16 pregnant females were checked daily for 1 week to detect the day of delivery. The first day pups were found in the cage was considered postpartum day 0. In the morning of postpartum days 2 and 4, pups were removed and the nest destroyed removing the nest material. After that, cotton pads were added. The number of pups per females varied from 2 to 5, and was not culled down to avoid unnecessary pup killing. Immediately after, all the pups were placed again into the mother cage. The following behaviors were scored for 15 min: time distant from pups (more than ~15 cm away), time spent licking and grooming the pups, time spent building a nest (gathering pieces of cotton pads around pups), latency to retrieve the first

pup, time crouching immobile over at least one pup (quiescent or passive crouching) or time crouching over the pups while doing other activities (active crouching). Quiescent or passive crouching was scored when the females spent more than 15 s immobile without interruption. These categories are based on the study of Stern and Johnson (1990) in rats, and adapted by Lonstein and DeVries (1999) for female and male voles.

Postpartum Maternal Behavior Test (Mother Undisturbed)

In the afternoon of Day 2 and 4, a 15 min behavioral observation was carried out without disturbing the mother. The time the mother spent licking and grooming, distant from pups, crouching immobile over pups (quiescent or passive crouching) or doing other activities (active crouching) was recorded. Quiescent crouching was scored when the females spent more than 15 s immobile without interruption.

Pups were left with their mothers until weanling age (20 days). After that, pups were weaned and dams housed individually for 30–45 days. Finally, all these females were placed in a clean cage, allowed to habituate, and tested one last time for their behavioral response to pups in a nonreproductive context.

Experimental Design for Selective Breeding

Sexually naïve, adult (60–90 days of age) male and female prairie voles were exposed to pups, tested for maternal responsiveness as described above, and categorized as being either maternal or nonmaternal (ignore or infanticide). Three generations of parental females and males; or nonparental females and males were bred. The first generation (G1) was formed in the following way: 8 mating pairs with random female and male trait (R–R); 6 pairs with maternal trait (M–M); and 8 with neglecting/infanticidal trait (NM–NM). At each generation, trait screening of the M, or NM behavioral response to pups in the offspring was carried out and only 10 pairs per trait were selected based on their behavior to produce the next generation (except for the random group). In the M–M line, only animals categorized as maternal were used. In the NM–NM line, only animals neglecting or attacking the pups were bred. In the R–R group, males and females were randomly assigned to partners irrespective of their behavior. We used some litter mates to continue the lines, but they were crossed to animals from different families. Breeders were chosen from different families, avoiding common grandparents to minimize inbreeding. Because infanticidal or neglecting males are very rare, we used some of the nonparental males more than once to sire multiple litters, but avoided crossing within the same family. Male responsiveness to pups was also selected in order to prevent the potential dilution of genetic factors, which may be common to both male and female parental care. However, males were removed from the cage before parturition. Because females and males share the caring activities, taking the male out of the cage intended to avoid the interference of the ''male factor'' in the study. In a variability study, males would significantly impact the behavior of females. Single breeding units have been previously used in our studies (Olazabal and Young, 2006) and are common in nature (Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993).

The random line served as control for stability of the proportion of NM and M traits in our colony. At each generation, responsiveness to pups in a nonreproductive context was quantified and animals were categorized as maternal or nonmaternal based on the criteria outlined above. The percentage of maternal and nonmaternal females among the groups was compared.

Statistical Analysis

All behavioral observations were scored manually by the author using the software Event Tracker (previous version of StopWatch). Proportional data were analyzed using Chi-square. ANOVA was used to analyze the main components of lactating maternal care (licking/grooming, active and passive crouching over pups, nest building, time distant from pups). ANOVA was followed with a post-hoc analysis (Fisher's PLSD).

RESULTS

Relationship Between Nonreproductive and Postpartum Maternal Behavior

Mother Disturbed. Females that displayed maternal behavior ($n = 6$), ignored/neglected ($n = 4$), or attacked ($n = 7$) pups in a nonreproductive context were paired with males for mating and their response to pups after delivery analyzed in detail. All groups displayed maternal behavior (retrieved, licked the pups, and crouch over them), and successfully reared their pups until weanling age. However, we found a few subtle differences. In the morning of the Day 2, females that attacked the pups spent less time ($F = 4.97$, $df = 2$, $p < .02$) crouching actively over the pups compared to those that ignored the pups or showed maternal behavior. The total time spent adopting crouching posture was not significantly different ($p = .16$) among the groups that attacked (553 ± 80), ignored (754 ± 72), or cared (679 ± 41) the pups. The time spent building a nest was different (Fisher post-hoc test, $p < .05$) between the females that previously attacked and those maternal, while females that ignored pups had intermediate values and did not differ significantly from the other two groups (Tab. 1). All females retrieved their pups and there was no difference among the groups that attacked, ignored or cared the pups in the other behaviors (Tab. 1). On the morning of the Day 4, the groups did not differ in any of the variables analyzed (data not shown).

Mother Undisturbed. The behavioral test on the afternoon of Day 2, with the mother undisturbed, did not reveal any significant difference among the groups. There was no difference in the time spent licking/grooming the pups (25 ± 19, 39 ± 17, 31 ± 12 s), and in active (174 ± 51,

Table 1. Maternal Behavior Test at Postpartum Day 2 (Mother Disturbed)

Group	Active Crouching	Passive Crouching	Latency to Retrieve 1st Pup	Licking	Distant From Pups	Building Nest
Maternal ($n=6$)	593 ± 13	87 ± 32	25 ± 13	84 ± 24	83 ± 40	305 ± 107
Ignore ($n=4$)	587 ± 31	166 ± 56	33 ± 17	65 ± 6	29 ± 28	118 ± 59
Attack ($n=7$)	$415 \pm 62^{*}$	138 ± 51	57 ± 29	70 ± 20	130 ± 38	76 ± 56^{a}

Data expressed as Mean $\pm$ SE seconds, $^{*}p < .05$, significantly different to the other two groups. $^{a}p < .05$ significantly different to the maternal group.

$333 \pm 81, 280 \pm 59$ s), passive ($704 \pm 85, 523 \pm 87, 619 \pm 58$ s) or the total crouching (878 ± 20, 856 ± 40, $899 \pm .05$ s) among the groups that attacked, ignored or cared the pups respectively. On the afternoon of Day 4, the results revealed that females that attacked pups spent more time in active ($F = 3.64$, $df = 2$, $p < .05$) and less in passive ($F = 4.65$, $df = 2$, $p < .02$) crouching than the other two groups (Fig. 1). However, there was no difference in the total time spent crouching over the pups between the females that attacked (801 ± 58 s), ignored (899 ± 0.4 s), or cared for the pups (898 ± 1.8 s). No other difference was found in the time licking and grooming the pups (51 ± 11, 31 ± 17, 38 ± 15 s), or time distant from pups (92 ± 61, 7 ± 7, 0 ± 0 s) among the females that attacked, ignored or cared the pups.

Finally, we averaged the scores for Days 2 and 4 in those equivalent variables that were not significantly different on each day. In that analysis females that attacked pups in a nonreproductive context spent more time distant from pups (morning and afternoon of Days 2 and 4 averaged) compared to females that ignored the pups or displayed maternal behavior ($F = 4.29$, $df = 2$, $p < .04$; Fig. 2). No other difference was found (data not shown).

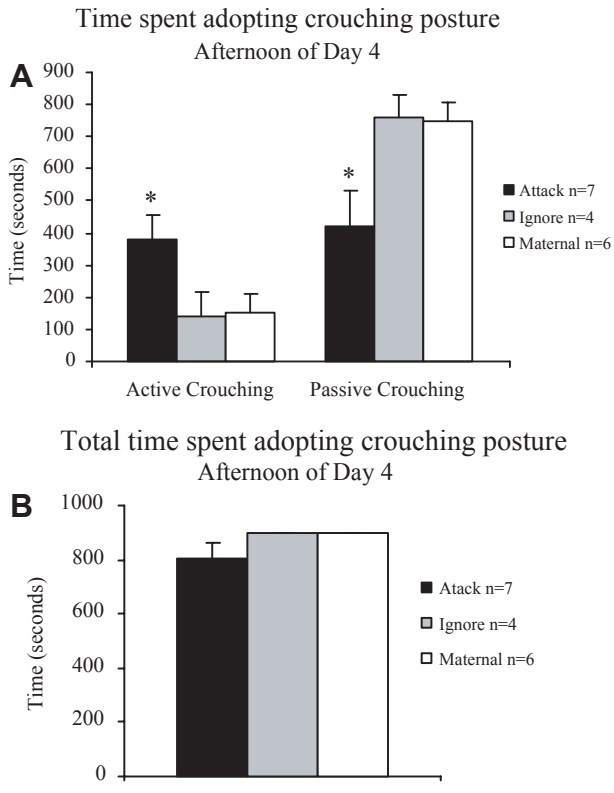

FIGURE 1 (A) Lactating females that attacked pups when tested in a nonreproductive context spent more time doing active crouching than females that showed maternal care or ignored the pups ($^{*}p < .05$). (B) Total crouching was not significantly different among the groups. The graph shows the average time spent by each group in a 15 min testing period on the afternoon of Day 4.

Stability of the Behavioral Response to Pups in a Nonreproductive Context After Postpartum Maternal Experience

Twelve females that displayed maternal behavior spontaneously and 20 females that either attacked (12) or ignored (8) the pups when tested in a nonreproductive context, were paired to males for mating, and the males removed 2 weeks later. After parturition, the females were not disturbed except to clean the cages and wean their pups at the age 20 days. One hundred percent of the females in

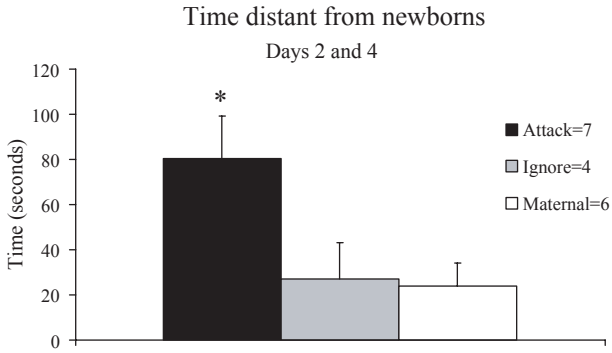

FIGURE 2 Lactating females that attacked pups when tested in a nonreproductive context spent more time distant from pups than females that ignored them or were maternal. Average of 15 min testing periods taken in the mornings and afternoons on postpartum days 2 and 4 ($^{*}p < .05$).

each group successfully reared their whole litter until weanling age. When these females were tested again 1 month after they had weaned their own offspring, they did not modify significantly their response to pups in a nonreproductive context, that is the maternal and nonmaternal groups were still significantly different (Chi-Square $p < .01$). Eighty percent (16/20) of the females that failed to show spontaneous maternal response as virgins failed to act maternally after 20 days of maternal experience (14 attacked and 2 ignored the pups; Fig. 3). In those females that were spontaneously maternal before parturition, 75% (9/12) maintained the same response to pups when tested again 1 month after weaning their own offspring, while one attacked the pups and two ignored them.

Potential Inheritance of Pup-Induced Behavioral Response in a Nonreproductive Context

We selectively bred maternal and nonmaternal animals for three generations. During the first two generations we did not find any significant difference (data not shown). When adult females from the third generation were tested, we found significant differences in the percentage of females that displayed maternal behavior in the different lines (Chi Square $p < .01$; Fig. 4). However, only the nonmaternal line was significantly different to the control and the maternal lines. Only 6% (one animal) of the females in the nonmaternal line showed maternal response when tested in a nonreproductive context. Control and maternal lines did not differ and displayed the normal variability in their behavioral response to pups, 56% and 55% of maternal versus 44% and 45% of nonmaternal females respectively. More subtle differences between the maternal females in the control and maternal lines were

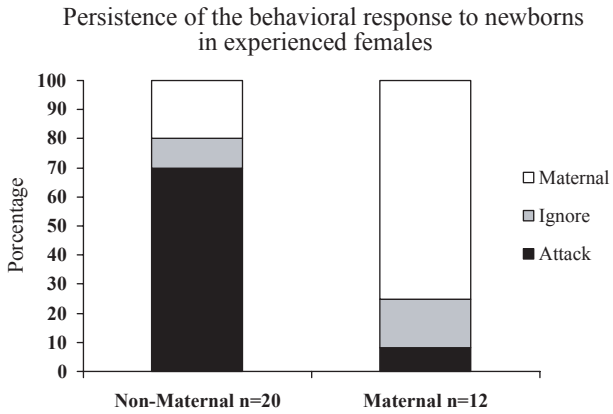

FIGURE 3 Most females that showed maternal care or failed to do so when tested as virgins in a nonreproductive context, maintained the same response after having 20 days of maternal experience as lactating mothers ($p < .01$).

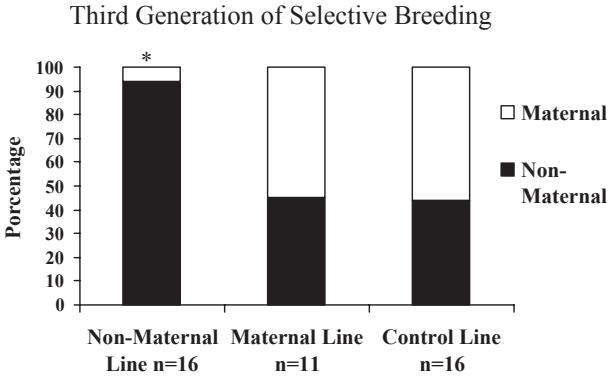

FIGURE 4 The female offspring of the nonmaternal line was significantly less maternal than the female offspring of the maternal and control lines (Chi square, $p < .01$). Animals represented in this graph are 16 descendent of 8 mothers of the control line, 11 descendent of 6 mothers of the maternal line, and 16 descendent of 8 mothers of the nonmaternal line.

investigated but there was not significant difference in the latency to retrieve pups, crouch over them or time spent building a nest (data not shown).

DISCUSSION

The present study investigated the spontaneous behavioral response displayed by female prairie voles when exposed to pups for the first time in a nonreproductive context. We contribute with evidence that (a) female prairie voles show significant stability in their behavioral response to pups, when tested outside the period of hormonal fluctuations typical of the end of pregnancy and parturition; (b) females that attack newborns in the naïve maternal behavior test show only subtle differences in the quality of maternal behavior during the postpartum period; (c) a single postpartum maternal experience does not reverse the failure to show maternal behavior in a nonreproductive context. Finally, (d) female prairie voles can be selectively bred for the absence of pup-induced maternal behavior.

We expected that naïve females that did not display maternal behavior after pup exposure would show significant differences in the quality of postpartum maternal behavior. However, our findings only show minor and subtle differences. For example, females that attacked pups were more active while crouching on the afternoon of Day 4 (undisturbed mother test), suggesting increased basal activity given that the test did not require any behavioral adaptation or response. In contrast, in the morning tests, the nest was destroyed intentionally and the mother had to rebuild it and retrieve or gather the pups. Under those conditions, females that were maternal in

previous tests showed higher engagement in nest building what might explain why maternal animals spent more time active during crouching without affecting the total time spent crouching over the pups. In addition, females that attacked pups spent higher average time distant from the pups on the period Days 2–4. It needs to be pointed out that most of the differences found in this study were not consistent for both days. Finally, the different number of pups per females (2–5) and the difference in nursing schedules of the lactating females might have increased the variability in some of the behavioral variables analyzed (i.e., active and passive crouching posture).

Considering these very subtle differences, our results seem to support the interpretation that parturition removed most behavioral differences found when the animals are tested as naïve in a nonreproductive context. The groups showed more similarities than differences for both days. However, it is possible that an increase in the time of behavioral testing on each day would reveal a more robust difference. Therefore we cannot exclude the possibility that infanticidal and maternal females behave slightly different during lactation and transfer some of these experiences to the offspring. Previous studies show evidence that subtle differences in the early environment can induce long-term effects on behavior (Champagne et al., 2001; Francis et al., 2000; Moore & Power, 1992; Moretto, Paclik, & Fleming, 1986).

Differences in activity levels and the time spent distant from pups among the groups might be consequence of different levels of anxiety or behavioral reactivity. In previous studies we found that females that attacked pups were less exploratory, and spent more time immobile in an open field, and isolated in a social interaction test than those that showed spontaneous maternal response (Olazabal & Young, 2005). Similar results have been reported by Clinton et al. (2007) using rats selectively bred for high or low reactivity to novelty. These authors found that females that were more exploratory and less anxious were more prone to show maternal behavior in a nonreproductive context.

The persistence in the failure to show maternal responsivity after experiencing postpartum maternal behavior was unexpected considering the strong evidence showing that past maternal experiences facilitate the induction of maternal behavior in rats (Bridges, 1977; Orpen & Fleming, 1987). The hormonal changes of the peripartum might induce a transient and drastic change in the behavioral response toward pups (Hayes & DeVries, 2007). Hayes and DeVries (2007), for example, propose a mechanism of facilitation of maternal behavior at parturition by distension of the birth canal. However, the memories established during that period of time are not sufficient to maintain maternal responses in a nonreproductive context, suggesting that is the behavioral response in a different biological and physiological context that persists in time. It is possible that those physiological events of the peripartum period do not have a long-term impact on reversing this behavioral phenotype. This interpretation, however, is based on the evidence found in the present testing conditions. We cannot exclude the possibility that after several deliveries and postpartum experiences, females would change their infanticidal responses. In addition it is possible that in other testing conditions, such as continuous exposure to pups, the same procedure might reveal experiential effects.

In the maternal behavior test in a nonreproductive context, the initial approach to pups is critical for the final behavioral output displayed by the subject. Emotional reactivity or neophobia can trigger infanticidal behavioral responses, before the animal can get used to pups. The subjects submitted to the single exposure to pups can be stressed by the very challenging testing conditions. As discussed above, a novelty seeking type of females would not be negatively stressed or aroused by the pups, then facilitating the approach to them and the induction of maternal behavior. Why male prairie voles, that rarely show infanticidal behavior, would not display that high emotional reactivity is unknown. Stowe et al. (2005) found that male prairie voles are less anxious and more exploratory than meadow voles, that agrees with their higher parental responsiveness. However Bales, Lewis-Reese, Pfeifer, Kramer, and Carter (2007), found that male and female prairie voles are not different in their exploratory behavior. Perhaps males and females show differences in their vulnerability to early stressors or other early developmental events that result in a subset of females developing higher emotional reactivity (Cushing, Yamamoto, Hoffman, & Carter, 2003; Yamamoto et al., 2004).

Finally, it is possible that a subset of females develops a different social strategy (reproductive competition) that triggers the aggressive behavior toward pups. How this difference would arise at laboratory conditions is unknown. However, it is important to note that differences in emotional reactivity and social strategies might be consequence of the development of hierarchies during housing conditions (Lanctot & Best, 2000). In those conditions, females can develop a different reactivity to the environment that influences their social and reproductive strategy, and transfer that information to their offspring through variation in maternal behavior. The presence of maternal responses in naïve females might be a condition to communal living groups in the field, while infanticidal females might be more prone to adopt solitary lives (McGuire, Pizzuto, & Getz, 1990).

The selective breeding experiment resulted in a rapid selection of a nonmaternal line. Although the maternal line did not show any improvement in the quality or frequency of maternal behavior, the nonmaternal line showed a significant difference in the number of nonmaternal animals compared to the control and the maternal lines. In particular the infanticidal behavioral response was predominant in this line. Many previous selective breeding experiments have found differences among the lines by the third generation, or even earlier (Brunelli et al., 1997; Stead et al., 2006; Touma et al., 2008). According to the findings of those authors we can be confident that prairie voles can be selectively bred for their behavioral response to pups in 3 generations. However, this selective breeding experiment was not continued until obtaining two consecutive generations what would strengthen our finding. These results also suggest that the nonmaternal is inherited more quickly than the maternal response. The rapid selective breeding of a nonmaternal line might explain why some laboratories have found that their colonies were mainly infanticidal (Lonstein & DeVries, 2001). Why infanticidal responses continue in the maternal line, even after negatively selecting this variable need to be investigated in further detail.

Recent findings suggest that rearing newborn prairie voles without the fathers reduce the occurrence of maternal behavior in the offspring (Ahern & Young, 2009). All our females in the three lines were reared without the fathers what might have potentiated the failure to show maternal behavior in the nonmaternal line, and maintained the regular variability in the others. This is also in agreement with a potential negative effect on spontaneous maternal behavior of leaving the pups unattended for longer period of time due to mother absence of the nest as found in the first experiment. Variability in the type of breeding units might be an interesting source of parental behavior variability in nature. According with the literature, more than a natural and breeding unit organization can be found in the field due to ecological and biological differences (Getz et al., 1993; Ophir, Campbell, Hanna, & Phelps, 2008). As a consequence the offspring might develop more o less prosocial and maternal behavior toward strangers showing more or less tendency to live in communal, pairs or single breeding units.

The development of two lines of prairie voles that differ in their behavioral response to pups can provide a good experimental model to test genetic, or epigenetic influences in parental behavior. Cross fostering studies (Stead et al., 2006) can also be very useful to discriminate between strong postnatal environmental effects (i.e., early maternal environment), or prenatal effects that are more independent of the postnatal environment. It would be of interest to investigate differences in genes expression in variables such as oxytocin receptor (see Olazabal and Young, 2006), and polymorphisms in two different lines or population of prairie voles that differ in their behavioral response to pups.

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