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Variability in “Spontaneous” Maternal Behavior is Associated with Anxiety-Like Behavior and Affiliation in Naïve Juvenile and Adult Female Prairie Voles (*Microtus ochrogaster*)

ABSTRACT: Juvenile female prairie voles (*Microtus ochrogaster*) are spontaneously maternal, while virgin adult females show significant variability in their response to first pup exposure, ranging from infanticidal to full maternal behavior. In the present study, we investigated whether differences in anxiety-like behavior and affiliation are associated with juvenile-adult and adult individual differences in the response to pups. Forty juvenile (19–20 days) and 42 adult (60–90 days) female prairie voles were exposed to pups for the first time and tested for maternal behavior, anxiety-like behavior (elevated plus maze, open field), and affiliation toward age-matched, same sex conspecifics. Juveniles displayed less anxiety-like behavior, were more affiliative to unfamiliar conspecifics, and interacted with pups more positively than adults. Adults that displayed maternal behavior spent less time immobile, made more crosses through the center of the open field arena, and were more affiliative than adults that attacked the pups. This suggests that lower locomotion or exploration in a novel environment and poor affiliative behavior are negatively associated with maternal responsiveness in female prairie voles.
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Prairie voles (*Microtus ochrogaster*) are a highly affiliative and parental species (DeVries, Johnson, & Carter, 1997; Getz, Carter, & Gavish, 1981; McGuire & Novak, 1984; Roberts, Williams, Wang, & Carter, 1998b; Thomas & Birney, 1979; Wilson, 1982; Young, Lim, Gingrich, & Insel, 2001). While juveniles from several rodent species are attracted to pups, they only act parentally after a few days of pup exposure (Bridges, Zarrow, Goldman, & Denenberg, 1974; Brunelli & Hofer, 1990; Lonstein & DeVries, 2000b; Mayer, 1983). In contrast,

most juvenile prairie voles (~20 days) display parental behavior (i.e., licking, grooming, and crouching postures) “spontaneously” on the first exposure to pups (Roberts, Miller, Taymans, & Carter, 1998a; Solomon, 1991; Wang & Novak, 1994). As shown in other rodent species (Mayer & Rosenblatt, 1979; Stern, 1987), female prairie voles appear to undergo a decline in their maternal response from weaning to adulthood (Lonstein & DeVries, 2000b, 2001). However, the quality of this behavioral change is still unclear.

While some authors report that the majority (64%) of adult female prairie voles, without prior exposure to pups (naïve), expressed maternal behavior spontaneously (Roberts et al., 1998a), others have described them as mostly non-maternal and infanticidal (Lonstein & DeVries, 2000a,b, 2001). This variability in the maternal response to pups in adult naïve females is also present

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across different experiments from the same laboratory as mentioned by Lonstein & DeVries (2001). These authors found that the percentage of adult females that acted maternally was quite variable and ranged from 18% to 50% of the females. What factors underlie variability in the maternal responsiveness to pups in female prairie voles is unclear and differences in geographic origin, photoperiod, early experiences or hormonal exposure have been proposed to be involved (Lonstein & DeVries, 2000a,b, 2001; Roberts, Zullo, Gustafson, & Carter, 1996; Roberts et al., 1998a,b).

Previous studies in rats have shown that differences in anxiety-like behavior, exploration, and fear may affect maternal response to pups. Hormonal regimes that facilitate maternal behavior reduce rat "timidity" in an open field apparatus (Fleming, Cheung, Myhal, & Kessler, 1989) and reduction of fear and anxiety by handling facilitate maternal behavior in rats (Mayer, 1983). Parturient females are also less neophobic, less anxious, and are more likely to enter and explore a novel environment than non-maternal females (Ferreira, Pereira, Agrati, Uriarte, & Fernandez-Guasti, 2002; Fleming & Luebke, 1981). Finally, juvenile rats, like parturient females, are less neophobic, more exploratory, and show less anxiety-like behavior in an open field than adults (Mayer & Rosenblatt, 1979; Moretto, Paclik, & Fleming, 1986; Smith & Morell, 2003). Despite all this evidence linking anxiety, fear, and exploration to maternal response, there is no evidence that population variability in anxiety, fear, or exploratory behavior in naïve females is associated with maternal responsiveness on the first pup exposure.

Adult rats overcome the initial avoidance of pups after several days of pup exposure (Fleming & Rosenblatt, 1974; Fleming & Luebke, 1981) and proximity to pups facilitates maternal behavior (Stern, 1997; Terkel & Rosenblatt, 1971). Reduced aversion to pups, or greater attraction to them, would increase the time near pups and facilitate the interaction with them, allowing maternal behavior to occur. This increased attraction to pups in juvenile rats may be part of an increased attraction to novel objects, social or non-social (Reiss, Smith, & Morell, 2003; Smith & Morell, 2003), but also part of a higher attraction specific for pups or young individuals as social partners (Mayer & Rosenblatt, 1979). In general, juvenile rodents are highly social and neither attack pups (Elwood, 1980; Gandelman, 1973) nor display serious aggressiveness toward other age-matched conspecifics (Delville, David, Taravosh-Lahn, & Wommack, 2003; Meaney & Stewart, 1981; Pellis, Field, Smith, & Pellis, 1997). Prairie voles are highly affiliative animals and show more interanimal contact, including time spent with pups, than two other closely related species such as meadow or montane voles (Salo, Shapiro, & Dewsbury,

1994; Wilson, 1982). Whether increased affiliative behavior or attraction to social stimuli in juvenile or adult rodents affect maternal response to pups is unknown, but the fact that spontaneous maternal behavior is common in female and male virgin prairie voles suggests that this may be the case.

Thus, like juvenile rats, juvenile voles are highly affiliative and interact with pups positively licking/grooming and crouching over them. As maturation occurs, a subset of female prairie voles goes on to become non-maternal or even infanticidal as adults, while other individuals maintain spontaneous maternal responsiveness as adults. The proportion of animals that show spontaneous maternal or infanticide behavior seems to vary even within a laboratory. Given the proposed relationship between anxiety-like behavior and affiliation with maternal behavior we hypothesized that: (a) juvenile-adult differences in maternal responsiveness in prairie voles would be associated with differences in anxiety-like behavior and affiliation; (b) naïve adult females that do not show maternal responses to pups would display more anxiety-like behavior, and less affiliation to conspecifics than maternal females.

METHODS

Subjects

Subjects were 42 adult (60–90 days of age) and 40 juvenile (19–21 days of age) naïve female prairie voles from our 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 our colony are derived from Illinois, field caught stock. Animals from the field were introduced last time in 1995. All animals were weaned at 19–21 days, maintained in same-sex (in general siblings) groups of 2–3 in cages 28 × 17 × 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-cobs[®] Laboratory Animal Bedding (Ohio) was used as bedding material. Cages in our animal colony are 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 maternal behavior test.

BEHAVIORAL TESTS

Maternal Behavior Test

Testing for all juveniles started the day following weaning and social housing. All females were moved to a new cage at ~1000 hr, 3 hr after the end of the dark phase, individually housed in a clean cage and allowed to habituate for 45–90 min before the maternal behavior test began. All observations were performed by the same experimenter with extensive experience in behavioral observations. The behavioral coders were also completely blind with respect to the animal's performance on previous tests. Maternal behavior test was always applied first because we wanted to exclude the possibility that applying other behavioral tests first may affect maternal performance. Counterbalanced order of behavioral test was avoided, so that each test was performed for each animal at the same time of day to avoid behavioral variation due to circadian rhythms.

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 approaches and withdrawals from the pups, latency to first approach, time far from pups (more than ~15 cm away), number of animals that attacks to pups, time spent licking and grooming, frequency and time spent building a nest (gathering pieces of cotton pads around pups), frequency of pup retrieval, latency to retrieve the first pup, time carrying pups, time hovering immobile over at least one pup (quiescence crouching), or time doing other activities (active crouching). Quiescence crouching was scored when the females spent more than 15 s immobile without interruption. A detailed posture observation as described by Lonstein and DeVries (1999) (high-low kyphosis, etc.) was not done in this study. Our criteria for considering an animal “spontaneously maternal” were that it licked the pups >5 s and spent >30 s adopting crouching postures over the pups. As previously reported by others (Lonstein & DeVries, 1999), retrieval was not included in the criteria given the low frequency of this behavioral component in prairie voles. Animals that neither reached the criteria for maternal behavior nor attacked the pups during the 15-min test period were categorized as females that “ignored” the pups. Pups were removed from the cage at the end of the test, or immediately after one of them was attacked by the subject, in order to avoid injury. Subjects that performed the attack were categorized as females that “attack” pups. Pups with serious injuries were euthanized immediately and those with a minimal wound were returned to their mothers after stopping the bleeding

and cleaning the wound. All pups were accepted by the mother upon returning to the nest.

Elevated Plus Maze Test

In the afternoon following the maternal behavior test, at ~1,400 hr, females were placed in the intersection of the open and enclosed arms (central platform) of an elevated plus maze raised 60 cm above ground with two open arms $50 \times 6 \times 0.6$ cm, and two enclosed arms $50 \times 6 \times 15.5$ cm extending from a common central platform (6×6 cm). Latency to enter the open arm, number of entrances into the open arm, time spent in the open arm, time spent in the central platform, number of entrances into the enclosed arm, and time spent in the enclosed arm were recorded during the 5 min session using a computer software (Stopwatch+; available to download at http://www.cbn-atl.org/research/cores/behavioral_software/stopwatch.cfm) specially developed to score behavioral observations. Eight juveniles and seven adults jumped or fell from the plus-maze and were excluded from the statistical comparison. The elevated plus maze was cleaned after each test with an Alconox[®] solution, and wiped with water.

Open Field/Locomotor Activity Test

Later in that afternoon, at ~1600 hr, females were placed in a $40 \times 40 \times 40$ cm transparent Plexiglas box. The behavior was recorded on videotape for 20 min, and the following behaviors were later scored manually: time spent in the center of the arena (a square of 18 cm), number of crosses through the center of the arena, time autogrooming, and time immobile. The animal's performance on the first and the second 10 min period was analyzed separately to determine differences during the initial highly active and exploratory 10 min and the second less active 10 min. The Plexiglas box was cleaned with an Alconox[®] solution after each test.

Female–female Affiliative Test

On the following day, animals were tested for affiliative behavior. Three clean cages connected by transparent Plexiglas tubes were used as the arena. The subject was placed in the central (neutral) cage and two other matched-age female conspecifics, one familiar and one unfamiliar, were tethered to the neck in each of the remaining cages. The experimental animal was free to move throughout the familiar or the unfamiliar female's cage, or to stay isolated from them in the neutral cage. The time the subject spent in each cage or in contact with the familiar or unfamiliar conspecific was scored using time-lapse recording of the 3-hr test. Tethered

animals were allowed to habituate to the cages at least 30 min before adding the subject and starting the test. Cages were deeply cleaned in our regular animal facilities after each test. A similar test to study female–female affiliation and male–female partner preference has been extensively used in our laboratory and by others (Lim, Wang, Olazabal, Ren, Terwilliger, & Young, 2004; Roberts et al., 1996). Only 35 juveniles and 35 adults of the initial subjects were submitted to this test for technical reasons, such as lack of same-sex siblings or familiar partners to perform the test.

The familiar partner was the animal with which the test subject was socially housed (typically a sibling). In adults, familiar conspecifics were sometimes non-related females housed together from weaning. No animal was isolated, but in many cases females were housed in groups of three. We avoided the use of experimental females as familiar or unfamiliar stimuli before they were tested in the affiliation test. For example, two of the animals that had been housed together were tested with the third being familiar. The female used as familiar stimulus twice was not tested as subject on this test but had been tested for maternal behavior, open field, and elevated plus maze. Unfamiliar females came from experimental animals that were not included in the experiment or experimental females that had already been tested once as subjects.

Statistical Analysis

Data were tested for homogeneity of variance (Bartlett's Test) and then analyzed by MANOVA (age or maternal categories—maternal-ignore or attack as factors) for selected variables in each behavioral test followed by Fisher's post hoc test for that specific number of variables. Regression analysis was applied to determine which measured variables better predicted maternal response. Data from selected variables from the open field and elevated plus maze was submitted to factor analysis using a principal component solution with an orthogonal rotation (varimax) of the factor matrix for more appropriate interpretation of anxiety data. Chi-square analysis was used to analyze frequency data. Statistical significance was $p < .05$. Data is expressed as means \pm SE.

RESULTS

Maternal Behavior

More adults (10 of 42, 24%) attacked the pups than juveniles (1 of 40; $\chi^2 = 8.01$, $df = 1$, $p < .01$), and more juveniles reached the criteria for maternal behavior (32 of 40, 80%) than adults (25 of 42, 59.5%; $\chi^2 = 4.05$, $df = 1$, $p < .05$). Seven juveniles and seven adults neither attack nor showed maternal behavior and they were categorized in this study as females that ignore pups.

There was a main effect for age on number of approach/withdrawals, licking/grooming, and carrying; $F(8,71) = 3.9$, $p < .01$. Juveniles showed less approach/withdrawal conflicts (2.4 ± 0.5 vs. 4.4 ± 1 ; $p < .03$), but there was no difference in the latency to approach to pups (114 ± 36 vs. 58 ± 24 s; $p = .36$). When only juveniles and adults that reached the criteria for maternal behavior were compared, adults showed more robust maternal behavior. Maternal adults retrieved pups more frequently and licked and groomed pups for a longer period of time than maternal juveniles (see Tab. 1). More adults built nests (16 vs. 2), and carried pups for longer period of time (7 ± 1 vs. 2 ± 1 s; $p < .01$) than juveniles. When juvenile and adult animals that retrieved one pup were compared, they did not differ in the latency to retrieve it (178 ± 53 s for juveniles vs. 118 ± 37 s for adults; $p = .35$). No difference was found in the amount of time juveniles and adults spent hovering over the pups both in the active or the quiescence posture (see Tab. 1). Juvenile and adult maternal animals spent similar amount of time far from the pups during the 15 min test period (83 ± 24 vs. 84 ± 24 s; $p = .98$).

Factor Analysis for the Open Field and Elevated Plus Maze

This analysis included four variables from the elevated plus maze (time open/closed arms, time in the central platform, number of entrances to the open arms, and total number of entries to arms) and from the open field/locomotor activity (crosses through center of the arena, time in center of the arena, and time immobile). Three factors emerged, accounting for 81.7% of the total

Table 1. Description of the Different Components of Maternal Behavior Displayed by Juvenile and Adult Female Prairie Voles

Age	Animals Retrieving one Pup	Animals Retrieving both Pups	Licking and Grooming (s)	Quiescence Crouching (s)	Active Crouching (s)	Animals Building Nest
Adult $n = 42$	17/42	9/42*	$273 \pm 29^{**}$	74 ± 22	517 ± 48	16/42**
Juvenile $n = 40$	10/40	2/40	155 ± 20	112 ± 24	429 ± 37	2/40

Data are expressed as frequency or means \pm SE.

* $p < .05$.

** $p = .01$. Licking/grooming and crouching include only maternal juveniles and adults.

Table 2. Orthogonal Factor loading for Three Open field and Elevated Plus Maze Variables

Variable	Factor 1	Factor 2	Factor 3
Open field			
Crosses through center		.87	
Time in center		.84	
Immobile		-.85	
Plus-maze			
Time open/enclosed	.89		
No. of entrances open arm	.93		
Time in central			
Platform			.93
Total entries	.77		.44

Factor loading of $<.3$ have not been included. The three factor account for 81.7 of the total variance.

variance (Tab. 2). The variables that loaded highly on Factor 1 were time open/closed arms and number of entrance to the open arms. On Factor 2 loaded all open field/activity box variables (time immobile loading negatively). Time in the center of the platform loaded highly on Factor 3. Total entries loaded modestly on both Factor 1 and 3.

Elevated Plus Maze

There was a main effect for age; $F(5,62) = 7.485, p < .01$. Juveniles made more entrances into the open arm than adults (31% vs. 22% of total entries; $p < .01$), and spent significantly less time in the central platform of the elevated plus maze than adults ($65.5 \pm 7.5, n = 32$ vs. 99 ± 8 s; $n = 35, p < .01$). However, there was no difference in the relative time (open/enclosed) spent in the open

versus enclosed arms in juveniles and adults ($.19 \pm .03$ vs. $.15 \pm .02$; $p = .24$), in the latency to enter the open arm (140 ± 32 vs. 149 ± 32 s; $p = .59$), or in the total entries into the arms (22 ± 2 vs. 19 ± 1 ; $p = .21$).

When adult animals were grouped according to their response to pups (maternal, ignore, attack), animals that attacked pups tended to spend more time in the central platform than maternal animals (maternal $85 \pm 12, n = 19$; ignore $107 \pm 18, n = 7$; attack $123 \pm 14, n = 9$), however, this result did not reach statistical significance ($p = .06$). No other difference in the performance in the elevated plus maze was found.

Open Field

There was a main effect for age; $F(4,75) = 2.69, p < .05$ for first 10 min and $F(4,75) = 4.7, p < .01$ for second 10 min). During the first and the second 10 min of the open field test, juveniles made more crosses through the center of the arena ($p < .05$), and spent less time immobile (second 10 min of test, $p < .05$) than adults (see Fig. 1). Juveniles also spent less time autogrooming (67 ± 9 vs. 117 ± 12 s; 68 ± 10 vs. 147 ± 15 s; $p < .01$) than adults. The time spent in the center of the arena was not different between juveniles and adults both during the first (20 ± 3 vs. 16 ± 2 s; $p = .27$) and the second 10 min (17 ± 3 vs. 10 ± 3 s; $p = .12$).

When adult animals were grouped according to their response to pups (maternal, ignore, attack), there was a group effect on the first 10 min of the open field, $F(4,36) = 3.4, p < .01$, but did not reach significant level on the second 10 min, $F(4,36) = 1.28, p = .29$. Post hoc tests showed that maternal animals made more crosses through the center and spent less time immobile than animals that attacked pups ($p < .01$; see Fig. 2). Animals that ignored the pups tended to perform as the animals that

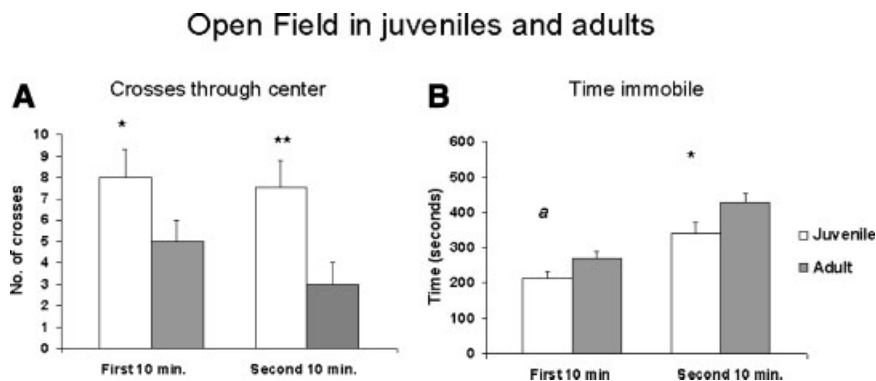


FIGURE 1 A: Number of crosses through the center of the arena during the first and second 10 min of the test. Juveniles made more crosses through the center than adults. B: Time spent immobile during the first and second 10 min of the test. Juveniles spent less time immobile than adults. Data expressed as mean \pm SE ** $p < .01$; * $p \leq .05$; $a, p = .055$.

Open Field in maternal and non-maternal adults

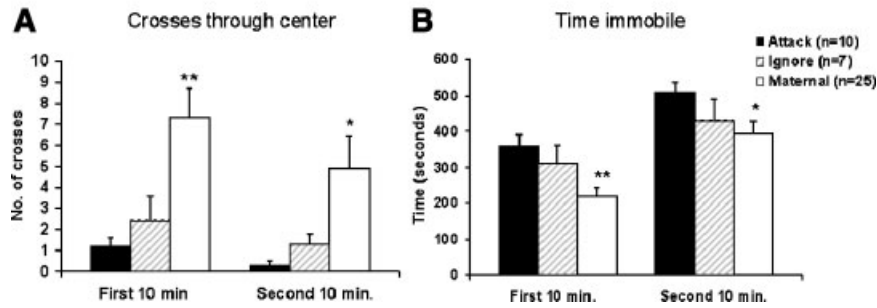


FIGURE 2 A: Number of crosses through the center during the first and second 10 min of the test. Maternal animals made more crosses through the center than animals that attacked the pups. **B**: Time spent immobile during the first and second 10 min of the test. Maternal animals spent less time immobile than animals that attacked the pups. Data expressed as mean \pm SE ** $p < .01$; * $p \leq .05$.

attacked the pups, but only the number of crosses through the center in the first 10 min reached a significance ($p < .05$) compared to maternal animals. There were no other significant differences between females that showed maternal behavior and animals that ignored the pups or between animals that ignore the pups and animals that attack them. No statistical differences among the groups were found in the time subjects spent autogrooming (maternal 105 ± 15 , ignore 119 ± 20 , attack 145 ± 26 s; first 10 min) or in the center of the arena (maternal 19 ± 3 , ignore 13 ± 7 , attack 10 ± 5 s, first 10 min; maternal 14 ± 4 , ignore 5 ± 2 , attack 5 ± 4 s, second 10 min) both in the first or second 10 min of the test.

Female–Female Affiliation

There was a main effect for age on time in social contact with conspecifics, time in the neutral cage and time in contact with the unfamiliar conspecific or in the unfamiliar's cage, $F(7,62) = 2.1$, $p = .05$). Juveniles spent more time in social contact with conspecifics (134 ± 5 vs. 107 ± 10 min; $p < .05$), and less time isolated in the neutral cage than adults (29 ± 4 vs. 61 ± 10 min; $p < .01$). Adults showed higher variability in the time spent in the neutral cage (see Fig. 3). Neither juveniles nor adults showed any preference for the familiar or unfamiliar conspecific, but juveniles spent more time in the unfamiliar's cage (90 ± 7 vs. 55 ± 10 min; $p < .01$) and in contact with the unfamiliar conspecific (79 ± 7 vs. 47 ± 10 min; $p < .01$) than adults.

In adults, there was a group effect for the affiliation test, $F(3,31) = 4.15$, $p = .01$). The post hoc test showed that adults that attacked the pups spent less time in social contact ($p < .02$) and more time isolated in the neutral cage ($p < .01$) than animals that ignored the pups or showed maternal behavior (see Fig. 4). There was no

difference in the time spent in the cage of the unfamiliar conspecific by animals that were maternal, ignored, or attacked the pups.

Predictive Value of Open Field Performance and Affiliative Test for Maternal Response

Regression analysis was applied to analyze the predictive value of open field variables and affiliative test for maternal response. Multivariate regression analysis revealed that number of crosses through center of the open field arena (first 10 min) and time spent in the neutral cage (affiliative test) were correlated to the maternal response, r of $.60$ ($p < .001$). More crosses through the center of the open field arena and less time spent in the neutral cage of the affiliation test were associated with a more positive response to pups.

When time spent adopting crouching posture (most distinctive component of maternal behavior) was related to the open field variables, we found a negative correlation between time adopting crouching posture and time spent immobile in the open field test (first 10 min). Simple regression analysis revealed that animals that spent more time adopting crouching posture tended to spend less time immobile in the open field test ($r = .47$, $p < .005$).

DISCUSSION

In this study, we show for the first time that population variability in maternal responsiveness is associated with variation in anxiety-like behavior and affiliation in virgin intact female prairie voles. We found that infanticidal behavior in virgin intact female prairie voles is associated with high anxiety-like behavior (more time immobile and fewer crosses through the center of the arena in the

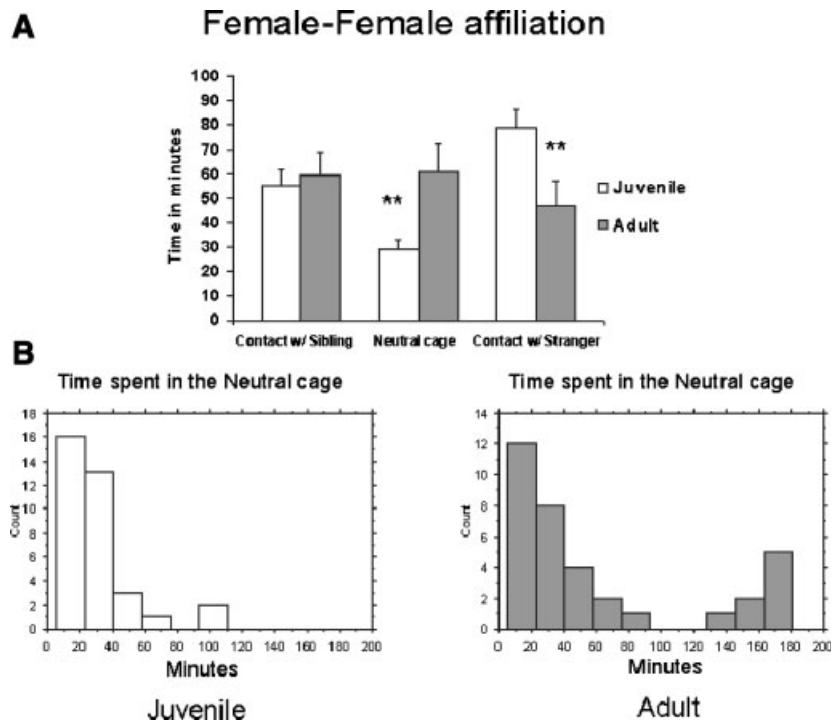


FIGURE 3 A: Time spent by juveniles and adults in the neutral cage or in contact with a strange or familiar age-matched same sex conspecific. Juveniles spent more time in contact with the stranger than adults. Data expressed as mean \pm SE $**p < .01$. B: Distribution histogram showing 10 intervals of time spent by juveniles and adults in the neutral cage.

open field) and low affiliative behavior to conspecifics (more time spent isolated in the neutral cage). Earlier studies have shown various relationships between anxiety and fear with maternal responsiveness in several species but, to our knowledge, this is the first to demonstrate a

relationship between variability in the first response to newborns and anxiety and affiliation within a population. Prairie voles are an excellent model to investigate this relationship because they show spontaneous maternal behavior, there is significant variability in the adult

Female-Female affiliation in maternal and non-maternal adults

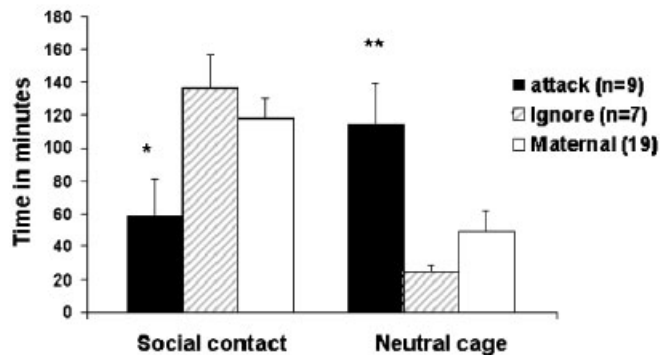


FIGURE 4 Time spent in social contact or in the neutral cage by animals that were maternal, that ignored or attacked the pups. Animals that attacked the pups spent more time in the neutral cage and less time in social contact with conspecifics compared to animals that ignored the pups or showed maternal behavior. Data expressed as mean \pm SE $**p < .01$; $*p \leq .02$.

response to pups, and they are highly affiliative compared to other related species. The present study also shows that factors underlying juvenile-adult behavioral differences in pup responsiveness may also explain adult variability in maternal responsiveness and infanticidal behavior.

It is important, for the appropriate interpretation of our data, to consider that all behavioral tests were performed in the same order. The maternal behavior test was always applied first, potentially affecting the performance of animals on the subsequent tests. For example, it could be argued that attacking pups during the maternal behavior test might influence the behavioral performance of the animals in the anxiety or affiliation tests. However, our data show that animals that ignored the pups were not different from those that attacked pups in the open field or the elevated plus maze. On the other hand, it is equally possible that less anxiety-like behavior and higher affiliation in maternal animals were not the consequence of the brief interaction with pups but a behavioral trait. Both possibilities are interesting and need to be investigated in future studies. However, the age differences that we found in prairie voles are similar to those found in rats applying single tests; suggesting that these differences are likely not consequence of the effect of previous tests.

Maternal Behavior

As shown by previous studies in prairie voles (Roberts et al., 1998a; Wang & Novak, 1994) and rats (Bridges et al., 1974; Brunelli & Hofer, 1990; Olazábal, Abercrombie, Rosenblatt, & Morrell, 2004; Smith & Morell, 2003), juveniles interacted more positively with pups than adults. Juvenile prairie voles showed less approach/withdrawal conflicts than adults before they started displaying "spontaneous" maternal behavior. The development of this approach/withdrawal conflict in rats has been previously associated with the higher incidence of attacks found in adult rats (Fleming & Luebke, 1981; Fleming & Rosenblatt, 1974).

This age decline in maternal responsiveness found in our study agrees with the findings of Lonstein and DeVries, 2000b, although in our case the decline was rather subtle and was based mainly in the increased number of females attacking pups. Excluding animals that attack pups, there is no difference in the number of animals showing maternal behavior in juveniles and adults. However, we do not imply that juveniles show higher quality of maternal behavior. When only maternal juveniles and adults were compared, adult maternal behavior was indeed more robust than juvenile maternal behavior. Adults licked and groomed pups for a longer period of time. While physical strength may affect the frequency of nest building and retrieval, it is unlikely that this can explain lower licking and grooming in juveniles.

The time spent hovering over the pups while performing other activities (licking, grooming, autogrooming, body posture adjustments), or hovering quiescently over at least one pup as described by Lonstein and DeVries (2001) did not differ in juvenile and adult females, suggesting that crouching sensory stimulation and motivation do not differ in juvenile and adult naïve prairie voles.

Principal Component Analysis for Open Field and Elevated Plus Maze

Previous studies show that different tests used to measure anxiety-like behavior and different variables usually measured in these anxiety tests reveal different aspects or facets of anxiety (Belzung & Le Pape, 1994; Cruz, Frei, & Graeff, 1994; Magara, Senent, Polo, & Magistretti, 2004; Pellow, Chopin, File, & Briley, 1985; Ramos, Mellerin, Mormede, & Chaouloff, 1998; Rodgers & Johnson, 1995). Factorial studies have shown that the concept of a single emotionality drive may be inadequate (Belzung & Le Pape, 1994; Cruz et al., 1994; Ramos, Berton, Mormede, & Chaouloff, 1997). For example, Magara et al. (2004) and Ramos et al. (1998) propose that locomotion and exploration in a novel environment vary independently from other measures of anxiety. More specifically, Ramos et al. (1998) proposed that locomotion in the center of the open field arena and the entrances to the open arm in the elevated plus maze may reflect two different types of emotional state. Although this group has found some inconsistency in their results (Ramos et al., 1997, 1998), our findings agree with that interpretation.

In our study, factor analysis of selected variables taken from the open field and elevated plus maze showed that time in the center of the open field arena, crosses through center of the arena and time immobile loaded all on the same factor with time immobile negatively correlated to the other two variables. Therefore, we will interpret these measures as representing locomotion and exploration in a novel environment. Time in open/enclosed arms or number of entrances to the open arm loaded on the same factor, in general associated with fear to height and open space (Cruz et al., 1994; Ramos et al., 1997, 1998; Rodgers & Johnson, 1995). Finally, time in the central platform of an elevated plus maze loaded on a separated factor associated to decision making/risk assessment (Cruz et al., 1994; Ramos et al., 1997, 1998; Rodgers & Johnson, 1995).

Elevated Plus Maze

The performance of juvenile prairie voles in the elevated plus maze (more entrances to the open arm and less time in the central platform) agrees with previous studies showing that juveniles show less anxiety-like behavior

and less conflict when confronted to a novel and fearful situation (Lopez-Rubalcava, Fernandez-Guasti, & Urban-Holmgren, 1996; Moretto et al., 1986; Smith & Morell, 2003). Several studies suggest that the time spent in the central platform of an elevated plus maze represents decision-making, probably related to approach/avoidance conflict (Cruz et al., 1994; Magara et al., 2004; Ohl, Toschi, Wigger, Henniger, & Landgraf, 2001; Rodgers & Johnson, 1995). The fact that our juveniles spend less time in the central platform, as they also show less approach/withdrawal conflict suggest that time in the central region of the elevated plus maze may also be a good predictor for maternal performance in adult prairie voles. In fact, in the present study, animals that attacked the pups tended to spend more time in the central region of the elevated plus maze than females that acted maternally. However, we failed to find differences in the number of entrances to the open arm or the time spent in the open arm among animals that were maternal, attacked, or ignored the pups. Although many studies found reduced anxiety in lactating females (Fernandez-Guasti, Ferreira, & Picazo, 2001; Lonstein, 2005; Toufexis, Rochford, & Walker, 1999), the elevated plus maze has not always been sensitive enough to detect changes in anxiety in lactating rats. Perhaps this is consequence of dissimilar testing conditions or differences in the response of different strain of rats (Ferreira et al., 2002; Fernandez-Guasti et al., 2001; Lonstein, 2005). In addition, the differences that our study in prairie voles and others in rats found in the performance of maternal animals in the elevated plus maze may be due to different hormonal background of our virgin female prairie voles and the lactating rat.

Open Field/Locomotor Activity

The lower anxiety-like behavior found in juvenile female prairie voles in the open field (more crosses through center of the arena, less time immobile, less autogrooming) also agree with previous studies in rats. Moretto et al. (1986) found that 20-day old juveniles show increased ambulation in the open field and emerge faster to the novel environment than adults, suggesting reduced emotional reactivity. As discussed above, our findings may specifically show higher locomotion and exploration in novel environment in juveniles compared to adults. Smith and Morell (2003) found that rats, 18 days of age, traveled longer distances and spent more time in the center of an open field than adults. This increased propensity to explore and the reduced anxiety or reactivity to novel environments in juveniles may increase the time in proximity with pups and let positive pup stimuli act to stimulate maternal behavior quickly. Brain correlates of this reduced reactivity to first pup exposure in juveniles have been recently found in rats. Juvenile rats show reduced or

no *c-fos* expression in the amygdala and the shell region of the nucleus accumbens (brain structures associated with anxiety and novelty) on the first exposure to pups (Olazabal and Morrell, manuscript submitted).

The differences between juvenile and adult performance in the open field were also very useful to predict adult variability in maternal responsiveness. In our study we found that adult prairie voles that ignored the pups or attacked them spent more time immobile and made less crosses through the center of the arena than maternal adults (more juvenile-like). Mayer and Rosenblatt (1979) hypothesized that an animal that is less adventuresome, and less active, may be less apt to interact with pups, and thus may require longer exposure before showing maternal behavior. Our findings agree with this hypothesis and with previous studies showing that manipulations that reduce fear, anxiety, or timidity such as lesions in the amygdala, or handling, among others, facilitate maternal behavior in adult naïve female rats (Fleming, Vaccarino, & Luebke, 1980; Fleming et al., 1989; Mayer & Rosenblatt, 1979).

Affiliative Test

Less attention has been put on the relationship between affiliative or prosocial behavior and maternal responsiveness. In the present study, affiliative behavior toward a conspecific was defined as seeking contact and social interaction with another individual when the animals have the option to freely avoid, stay close but not in contact, or interact with conspecifics. The three cage paradigm used in this study showed that juvenile prairie voles, as previously proposed by others in rats (Mayer & Rosenblatt, 1979), are attracted to same age individuals and spend more time interacting with them than adults. However, adults showed significantly higher variability in their performance in this test than juveniles.

The higher time spent by juveniles in contact with the unfamiliar or in the cage of the unfamiliar conspecific and the lower time spent in the neutral cage agrees with the reduced fear to novel social or non-social stimulus found by previous studies in juvenile rats (Mayer, 1983; Smith & Morell, 2003). However, in contrast to what was found in other studies (Roberts et al., 1996; DeVries et al., 1997) adult females did not show preference for the familiar animal. However, the critical aspect of this same-sex affiliative test, in this study, was to quantify the time spent interacting with another conspecific (regardless of their preference) versus isolated in a neutral cage. What factors underlie the increased time spent in the neutral cage in a small subgroup of adult females can be speculated upon. Higher hostility or reactivity to the conspecific stimuli may have induced these animals to avoid the conspecific rather than socially interact with

them. However, the possibility that the behavior of the other two conspecifics influenced the behavior of the subject cannot be excluded.

Emotional reactivity has been proposed to contribute to the modulation and variation of aggressive behavior (Delville et al., 2003). Juvenile rodents are in general less reactive to novel stimuli (Mayer, 1983; Smith & Morell, 2003), rarely attack pups (Gandelman, 1973; Lonstein & DeVries, 2000b; Mayer, 1983) and are in general less aggressive than adults (Delville et al., 2003; Meaney & Stewart, 1981). While play-fights are described as spontaneous events in 20–30 days juvenile rodents, play-fights in older animals are generally initiated by one animal and lead to almost immediate domination of the other one (Meaney & Stewart, 1981; Delville et al., 2003; Pellis et al., 1997). In our study, adult females that were more juvenile-like in terms of their exploration and affiliative behavior were also less prone to attack pups. Maturation of agonistic behavior, reduced prosocial behavior, and increased emotional reactivity may affect maternal response in female prairie voles and explain why more adults attack pups. Previous studies in mice (Parmigiani, Palanza, Rodgers, & Ferrari, 1999) found that intrasexual aggression and infanticide behavior are related and share similar function (i.e., competition for mates and resources). What factors underlie the differences in reactivity and affiliative behavior in adult prairie voles are unknown. However, previous studies suggest that early experiences, including social experiences, may affect emotional reactivity, anxiety, and aggressive behavior (Caldji, Tannenbaum, Sharma, Francis, & Plotsky, 1998; Champagne, Diorio, Sharma, & Meaney, 2001; Delville et al., 2003; Huck, Soltis, & Coopersmith, 1982; Pellis et al., 1997).

Despite the observation that adults were less affiliative than juveniles, this reduction in affiliation was mostly due to a subgroup of adult animals. Only a small subgroup of adults showed very low affiliation to conspecifics and tended to attack pups. Studies performed in seminatural conditions and natural populations appear to support this finding. Thomas and Birney (1979) found that about one third of their prairie voles were asocial. Asocial behavior in prairie voles was also associated, in their study, with lower survival probability and low reproductive success. These authors found that asocial prairie voles often were hiding, presented wounds, and eventually died mostly around the beginning of the reproductive stage. According to Thomas and Birney (1979), this may be due to intrasexual competition. Whether our females housed together after weaning establish some competitive or hierarchical social interaction affecting their later performance is unknown but will be investigated in future studies.

Studies in free-living prairie voles show that social organization varies significantly in this species (Getz &

Hofmann, 1986; Getz, McGuire, Pizzuto, Hofman, & Frase, 1993). Three breeder units are common in free-living prairie voles: male-female pairs with offspring (monogamous); single reproductive female; and complex units or communal groups that included more than one adult male or female with offspring. This suggests that the mating system and social and reproductive behavior vary in these animals, affecting their adaptability to the different environmental or social situations encountered in their lives. Whether changes in the response to pups also vary in natural populations is unknown. Although comparison between data from free-living and laboratory animals is important to understand the biology of the behavior studied (Wolff, 2003), data about incidence of infanticidal behavior in free-living animals is difficult to obtain. However, since about 50% of all breeding units receive eventual visits from unrelated females (McGuire, Pizzuto, & Getz, 1990; Getz & Hofmann, 1986), there are opportunities for the occurrence of infanticidal behavior when mothers are not present in the nest. Data from field studies show that single reproductive units are the most visited by unrelated females (McGuire et al., 1990). On the other hand, complex breeding units or communal groups (Getz & Hofmann, 1986) would require a positive maternal interaction with pups of other females and the inhibition of infanticidal behavior. How our laboratory females developed this variability in infanticidal behavior through development will be focus of future studies.

Despite the strong association found in our study between anxiety-like behavior and affiliation and infanticide behavior, other factors must be affecting the maternal response. The variability in pup killing in the different species and from experiment to experiment has been a common phenomenon in rodents (Jakubowski & Terkel, 1985; Lonstein & DeVries, 2000b) and the reasons for this are still unclear, but genetic, hormonal, and social factors are known to influence infanticidal behavior (Brown, 1986; Lonstein & DeVries, 2001; Numan & Insel, 2003). We are currently investigating whether females that showed differences in maternal response to pups show also differences in the density of receptors for oxytocin and vasopressin in the brain. Previous studies in our laboratory showed that there is a significant variability in oxytocin and vasopressin receptor expression in the prairie voles' brain (Phelps & Young, 2003; Young et al., 2001). Oxytocin and vasopressin are two related peptides that have been previously involved in parental behavior, affiliation and anxiety in voles and other species (Bielsky, Hu, Szegda, Westphal, & Young, 2004; Lim et al., 2004; Wang, Liu, Young, & Insel, 2000; Numan & Insel, 2003; Wigger et al., 2004; Young et al., 2001). These studies could provide a neurochemical correlate for individual differences in anxiety, affiliation, and maternal responsiveness.

NOTES

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