Original Paper

Brain, Behavior and Evolution

Brain Bevah Evol 2008;71:32–40
DOI: 10.1159/000108609

Received: February 2, 2007
Returned for revision: April 29, 2007
Accepted after second revision: June 28, 2007
Published online: September 20, 2007

Female-Directed Aggression Predicts Paternal Behavior, but Female Prairie Voles Prefer Affiliative Males to Paternal Males

Alexander G. Ophir[a] Ondi L. Crino[a] Quiana C. Wilkerson[a] Jerry O. Wolff[b] Steven M. Phelps[a]

[a]Department of Zoology, University of Florida, Gainesville, Fla., [b]Department of Biological Sciences, St. Cloud State University, St. Cloud, Minn., USA

Key Words
Affiliation · Aggression · AVP · Mate choice · *Microtus ochrogaster* · Monogamy · Pair bond · Paternal care · V1aR · Vasopressin

Abstract
In the socially monogamous prairie vole, *Microtus ochrogaster*, male affiliation and parental care are influenced by the neuropeptide arginine vasopressin and expression of its receptor V1aR. If parental care and adult affiliation can be considered a behavioral syndrome, females might use male affiliative behavior as a cue to choose a good father. We investigated three questions: (1) do females prefer affiliative males; (2) do males that are affiliative with females demonstrate paternal behavior with pups; and (3) is male V1aR expression related to male behavior or female preference? We evaluated paternal behavior of individual males, then offered sexually receptive females a choice between paternal and non-paternal males and measured the proportion of time each male spent engaging in affiliative behavior with the choosing female. Females showed a preference for more affiliative males, but affiliation was not predictive of paternal care. Thus females did not discriminate between paternal and non-paternal males. Perhaps surprisingly, paternal behavior was correlated with the relative amount of aggression males directed toward females. Finally, females did not discriminate between males with high or low V1aR expression and V1aR expression did not predict male affiliative behavior or parental care. These data suggest that male affiliative behavior, but not paternal care, is associated with female mate choice.

Introduction

One leading hypothesis for why monogamy should evolve is that individual fitness is increased from the additional parental care of a second caregiver [Wittenberger and Tilson, 1980; Clutton-Brock, 1989]. For example, many bird species exhibit this added benefit of social monogamy [e.g., Lack, 1968; Orians, 1969]. Although monogamy is quite rare in mammals [Kleiman, 1977], bi-parental care seems to increase fitness in some carnivores [e.g., Moehlman, 1989], primates [e.g., Goldizen, 2003; van Schaik and Kappeler, 2003], and rodents [Gubernick et al., 1993; Gubernick and Teferi, 2000]. In some fishes, paternal care serves as a basis for female mate choice [Petersen, 1995; Wiegmann and Baylis, 1995; Östlund and Ahnesjö, 1998; Pampoulie et al., 2004]. Similarly in monogamous mammalian species with bi-parental care, females may select mates that possess and advertise cues indicating paternal behavior. The prairie vole *(Microtus*

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/bbe

Alexander G. Ophir
618 Carr Hall, Department of Zoology
University of Florida, PO Box 118525
Gainesville, FL 32611 (USA)
Tel. +1 (352) 392 6212, Fax +1 (352) 392 3704, E-Mail aophir@ufl.edu

ochrogaster) is socially monogamous, forms long-term partnerships, and exhibits paternal care [Thomas and Birney, 1979; Gavish et al., 1981; Getz et al., 1993]. Given that prairie voles are unlikely to form pairs after the loss of a partner [Pizzuto and Getz, 1998; Thomas and Wolff, 2004], females presumably have only one chance to choose a mate, and therefore ought to select males carefully. Females might therefore base mate choice decisions on male affiliation if such affiliative behavior indicates subsequent paternal affiliation.

One potential source for phenotypic correlations among affiliative behaviors arises from the shared role of vasopressin and its primary central nervous system receptor (V1aR) in pairbonding [Winslow et al., 1993; Young and Wang, 2004] and parental care [Wang et al., 1994a]. Because the mechanisms of pairbond formation and paternal care are linked, females might be able to use phenotypic correlations between behaviors to select paternal males. If general affiliation in male prairie voles comprises an overarching behavioral syndrome [sensu Sih et al., 2004], then a positive correlation should be observed between affiliation with prospective mates and with pups. Furthermore, if such a behavioral syndrome does indeed exist, females might use male affiliative behaviors demonstrated during the initial stages of pairbond formation as a cue of their potential quality as fathers. Moreover, V1aR expression might reflect the effects of selection and predict which males are preferred, which males are most paternal, or both.

We investigated the potential for a mechanistically related behavioral syndrome encompassing general affiliative behavior. We used a series of behavioral tests to ask if females prefer affiliative males, if affiliative males are also paternal, and if V1aR expression predicts paternal behavior or is associated with female preferences. Males were screened with 3 to 5 day old pups to assess the degree to which they were naturally paternal. We then offered receptive nulliparous females a choice between high and low paternal males to see if females preferred paternal males. Finally, we examined V1aR expression in these males, to see if expression in neural areas associated with pairbond formation or paternal care correlated with affiliative or paternal behaviors.

Materials and Methods

Animals

All individuals were second or third generation offspring, derived from routinely outbred wild-caught populations from Champaign County, Illinois and were housed at the University of Florida. At weaning, we grouped all animals into same-sex littermates and maintained them under a 12:12 L:D cycle in polycarbonate cages (47 × 25.5 × 15.5 cm). No animals used in this experiment were reared in isolation. Food and water were provided ad libitum and temperature was maintained at 21 ± 2°C. The experiments discussed below were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, project number D289, and were in accordance with the guidelines set by the UF Animal Research and Care Committee.

Procedure

All animals used were sexually mature, but inexperienced, adults similar in size, weight, and age (80 to 200 days old). This study consisted of two tests: paternal care test and choice test.

Paternal Care Test. We screened 32 adult virgin males for paternal care. A test began by placing a male into a clear Plexiglas box (49 × 28 × 23 cm) and allowing him to acclimate to the apparatus for 10 min. After the acclimation period an unrelated 3–5 day old pup, taken from an established breeding pair, was placed in the corner of the apparatus farthest from the male for 10 min. Under such circumstances, male prairie voles exhibited behavior ranging from accepting pups as their own to attempting infanticide. We used a digital video camera (Sony Handycam DCR-HC20) connected to a video recorder (Panasonic AG-6040), to document the males' behavior for later analysis. Trials were immediately terminated if males attacked the stimulus pup and these males were classified as non-parental. Non-parental males were given a score of 1, indicating they attacked a pup; males that did not attack pups were given a score of 0. A naïve independent observer analyzed the video recordings using JWatcher (v.1.0), and scored the time a male licked/groomed, huddled/crouched, or carried the infant and whether or not they attacked the pup. Using these measures we sorted males by the amount of paternal care they demonstrated and selected males from the upper and lower quartiles to serve as stimulus males in the subsequent choice test. Thus males demonstrating a high degree of licking/grooming, huddle/crouch, and carry behavior and no attacking were termed 'parental' males (PAR; n = 8), whereas males that attacked pups were termed 'non-parental' males (NPAR; n = 8).

Choice Test. Eighteen adult nulliparous females were exposed to the soiled bedding of a reproductively active adult male for 3 days before the test to induce sexual receptivity [Hofmann and Getz, 1988]. Because we limited our stimulus males to eight PAR males and eight NPAR males, we reused individual males for multiple trials (see below). We anticipated that some males would mate during the choice test. Therefore, we controlled for mating behavior by ensuring that all males had mated three weeks prior to use in a choice test. Lastly, to avoid issues of pseudo-replication we assigned males of each category to all 120 possible pairs and then randomly selected 18 unique pairs to serve as a stimulus for each trial. Out of necessity, males were re-used across trials. However, when males were reused, we ensured that at least three weeks separated one trial from the next and all males served in at least one and no more than three trials.

To begin a choice test, males serving as either a PAR or NPAR stimulus male were tethered in one of two separate 'holding' chambers in a three-chambered apparatus following the design and methods of Wolff and Dunlap [2002]. PAR and NPAR males

were counterbalanced for side from one trial to the next. Females were introduced into the third 'neutral' zone of the apparatus and all behavior was recorded on a time-lapse video recorder for 24 h. A naïve independent observer, blind to male type, then analyzed the videotapes for each trial using JWatcher. We recorded the amount of time females spent in each holding chamber, spent in side-by-side contact with each male, and mated with each male. Next we measured the time that each stimulus male and female allogroomed each other. Finally, we recorded the amount of time either stimulus male directed aggression (including lateral threats, lunges, chases, bites, and upright posture) toward the female to assess the degree to which males were receptive to female approaches. We defined the preferred male of a trial to be the stimulus male with whom the female spent at least 25% more time in side-by-side contact; the non-preferred male was consequently the male with whom the female spent less time in side-by-side contact.

Neuroendocrine Comparison

After all choice tests were completed we collected the brains of the males serving as stimulus animals by euthanizing subjects with CO_2 followed by rapid decapitation. Once dissected, the brains were frozen on powdered dry ice and stored at –80°C until sectioning. Four sets of coronal slices (20 μm thick at 100 μm intervals) were mounted on superfrost slides (Fisher Scientific), and stored at –80°C. We used standard protocols for autoradiography using ^{125}I-linear-AVP (Perkin-Elmer) to visualize and quantify V1aR binding [Insel et al., 1994; Young et al., 1997] on one of these sets. This ligand binds strongly and specifically to vole vasopressin receptors [Wang et al., 1997], has comparatively high affinity for V1aR (55 pM) over other related receptors [Johnson et al., 1993], and has revealed species differences in binding between prairie voles and montane voles *(M. montanus)* corresponding to V1aR mRNA expression [Young et al., 1997] confirming ligand specificity. To process tissues, we lightly fixed sections in 0.1% paraformaldehyde, washed them in 1× Tris, incubated them with 50 pM ^{125}I-linear-AVP for 60 min, washed them again in 1× Tris with $MgCl_2$, and finally rapidly air-dried them. We then exposed the radioactive sections to film for 72 h alongside autoradiographic standards. High expression of V1aR results in high binding of radioactive ligand, and can be measured by the optical density of film exposed to the tissue sections. We investigated V1aR across all forebrain structures known to exhibit specific binding [e.g., Insel et al., 1994; Phelps and Young, 2003] by digitizing films on a Microtek Scan Maker 5900 and quantifying the standardized scans using NIH Image J software. Optical density measurements were converted to decompositions per minute per milligram tissue equivalent based on autoradiographic standards. Specifically, we focused on quantifying V1aR expression in the ventral pallidum (VP), the lateral septum (LS), bed nucleus of the stria terminalis (BST), and the medial and lateral dorsal thalamic nuclei (MD Thal and LD Thal; fig. 1). We chose these areas because they express V1aR [e.g., Young et al., 2001; Phelps and Young, 2003], play a role in the reward circuit and social memory, and/or are integrally involved in male pairbond formation, paternal care, or both [e.g., Bamshad et al., 1994; Wang et al., 1994a; Young et al., 1997; Liu et al., 2001; Lim et al., 2004]. Nonspecific binding was estimated from background levels of cortical binding on the same sections [Young et al., 1997].

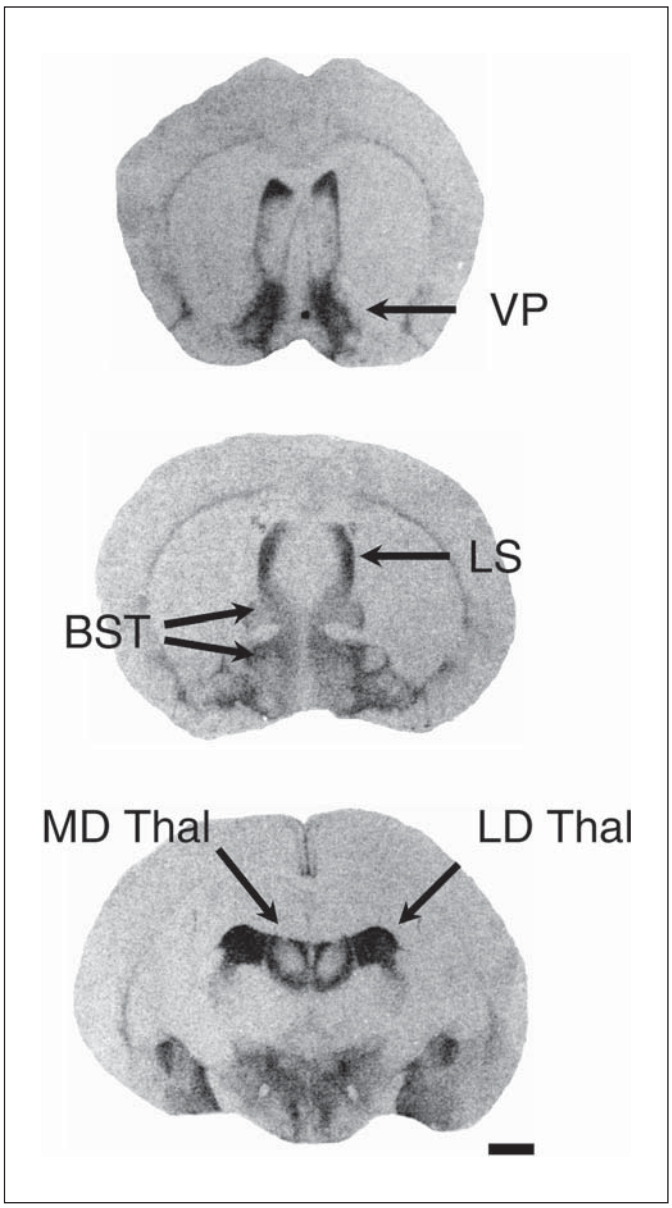

Fig. 1. V1aR expression in coronal sections of prairie vole ventral pallidum (VP), lateral septum (LS), bed nucleus of the stria terminalis (BST), medial dorsal thalamus (MD Thal), and lateral dorsal thalamus (LD Thal) using ^{125}I-linear AVP radio-labeled ligand. Scale bar, 1.0 mm.

Statistics and Analysis

To investigate if male behavior in one context predicted behavior in another context we analyzed data using Pearson's correlation coefficients and determined significance using Fisher's r-to-z conversions. Specifically we asked if paternal behavior was correlated with affiliative behavior or aggressive behavior or if V1aR expression correlated with measures of paternal care or affiliation. For these comparisons each trial was considered the independent unit for comparison (see below).

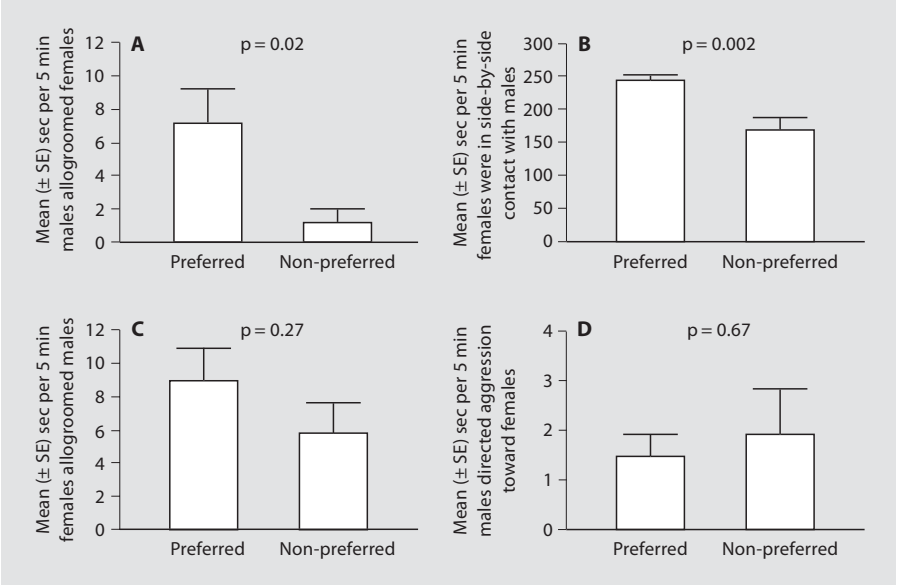

Fig. 2. Mean (± SE) proportion of time preferred and non-preferred stimulus males (**A**) allogroomed females, (**B**) spent in side-by-side contact with females, (**C**) were groomed by females, and (**D**) directed aggression toward females.

These analyses were complicated by the fact that, by definition, females spent more time with preferred males than non-preferred males and males were reused out of necessity. The total amount of time a female spent in each male's chamber varied considerably within each trial and represents the upper limit of time that a male had access to the female. The amount of time a male had access to a female should increase directly with the amount of time he engages in a given behavior (affiliative or otherwise). Therefore, rather than comparing the absolute mean time males engaged in affiliative behaviors with females, we normalized male-female interactions between a pair of males by comparing the proportion of time they interacted with females. Comparing between males using the proportional time each engaged in a particular behavior accounts for this potentially confounding correlation.

Proportions were calculated by dividing the amount of time a male engaged in a given behavior (e.g., the affiliative or aggressive behaviors listed above) by the maximum time a particular male was able to engage in those behaviors (measured by the time the female resided in that male's chamber). To ease interpretation, we converted these proportions into seconds per 5 min by multiplying all values by a constant (300). Finally, we took the difference between the preferred and non-preferred males to avoid pseudoreplication due to re-using males. This provided an independent datum for each trial, with positive numbers reflecting higher values for preferred males and negative numbers reflecting higher values for non-preferred males.

We also asked if preferred and non-preferred males differed behaviorally or in their neural V1aR expression. Individual males were either preferred or non-preferred consistently across trials. Therefore, to compare behavioral phenotypes of preferred and non-preferred males we compared the mean proportional time each male engaged in affiliative, mating or aggressive behavior using Student's t tests. Similarly, we compared V1aR expression between preferred and non-preferred males using Student's t tests. For all analyses we considered differences at the $p \leq 0.05$ level significant.

Table 1. Absolute amount of time females spent interacting with preferred and non-preferred males

	Preferred	Non-preferred
Total time in chamber, min	151.43 ± 10.52	48.21 ± 8.10
Side-by-side contact, min	121.68 ± 9.29	29.14 ± 5.96
Males groomed females, min	3.83 ± 0.96	0.14 ± 0.10
Females groomed males, min	4.34 ± 0.96	1.03 ± 0.35
Mating, min	8.23 ± 3.78	0.24 ± 0.24
Female-directed aggression, min	0.62 ± 0.27	0.29 ± 0.11

Means ± standard errors are presented for preferred and non-preferred males in each trial (n = 15). Data were not analyzed as within pair comparisons due to issues of pseudoreplication and non-independence between trials.

Results

In the choice test, we analyzed the 15 trials for which we had behavioral data; we excluded three trials from the analysis due to technical problems. Furthermore, two stimulus males unexpectedly died before their brains could be taken. Consequently, we were unable to include these data in our analyses for the three trials in which at least one of each of these males served.

Do Females Prefer Affiliative Males?

Data analysis was conducted on proportional data as described above, however actual times are presented in table 1 for reference. Preferred males spent proportion-

Table 2. Correlation matrix for affiliative behaviors from the choice test and paternal behaviors from the paternal care test

	Proportion of time in side-by-side contact	Proportion of time males allogroomed females	Proportion of time spent mating
Lick/groom pup	r = –0.26; p = 0.44	r = 0.02; p = 0.96	r = 0.03; p = 0.93
Huddle/crouch pup	r = –0.20; p = 0.58	r = –0.22; p = 0.53	r = 0.25; p = 0.48
Carry pup	r = –0.54; p = 0.09	r = 0.05; p = 0.90	r = –0.04; p = 0.91
Attack score	r = 0.28; p = 0.42	r = 0.10; p = 0.78	r = –0.11; p = 0.76

Values were calculated as the differences between preferred male minus non-preferred male. All p's were calculated using Fisher's r-to-*z* conversion. n = 11 for all correlations listed.

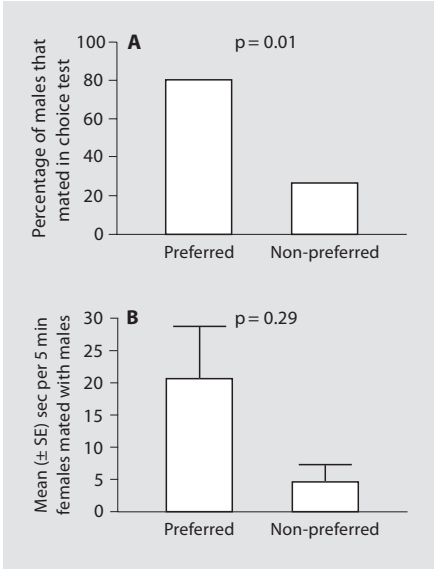

Fig. 3. A The percent of preferred or non-preferred males that females mated with across trials (n = 15). **B** Of mated males, the mean (±SE) proportion of time females spent mating with preferred (n = 7) and non-preferred (n = 4) males.

ately more time allogrooming and in contact with females than non-preferred males (Student's t test: Male to Female Allogroom, $t_{(14)} = 2.74$, p = 0.02; Contact, $t_{(14)} = 3.94$, p = 0.002; fig. 2A, B). In contrast, females spent proportionally similar amounts of time allogrooming preferred and non-preferred males ($t_{(14)} = 1.15$, p = 0.27; fig. 2C). Females mated with 12 of 15 (80.0 %) preferred males and 4 of 15 (26.67%) non-preferred males (Fisher's exact test: $p < 0.01$; fig. 3A). The four females that mated with the non-preferred males also mated with the preferred males. For the males that mated in those 15 trials, we found no significant difference in the amount of time preferred and non-preferred males mated ($t_{(14)} = 1.10$, p = 0.29), however preferred males tended to mate with females longer (fig. 3B). The proportional amount of aggression directed toward females did not differ significantly between preferred and non-preferred males ($t_{(14)} = 0.44$, p = 0.67; fig. 2D). Together these data demonstrate that not only do females prefer male affiliation, but that female-directed affiliation is independent from female-directed aggression and females ignore male aggression when selecting mates.

Are Affiliative Males Paternal?

Parental males (PAR) licked and groomed pups for a mean of 358 (± 96.21 SE) s, and huddled and crouched with pups 144.2 (± 101.51) s. Eight of 8 NPAR males were from the lower quartile and attempted infanticide. Screening trials involving NPAR males were terminated immediately after an attack was initiated, which was often within the first minute of the trial. Finally, no measures of affiliation predicted any aspect of paternal behavior (table 2).

Is Paternal Behavior Predicted by Other Male Behavior?

We did not find a relationship between affiliation with females and paternal care. However, preferred males that were more aggressive with females in the choice test spent more time licking and grooming pups (Pearson's correlation: r = 0.75, n = 11; Fisher's r-to-z conversion: p = 0.006), huddling and crouching with pups (r = 0.59, n = 11, p = 0.05), and carrying pups (r = 0.81, n = 11, p = 0.002; fig. 4A, C). Furthermore, preferred males that were more aggressive with females in the choice test were less likely to have attacked pups (r = –0.75, n = 11, p = 0.006; fig. 4D). Taken together, preferred aggressive males were more paternal, whereas non-preferred aggressive males were less paternal. Female-directed aggression alone, however, was not a predictor of mate choice. A comparable number of preferred males were either high or low paternal males

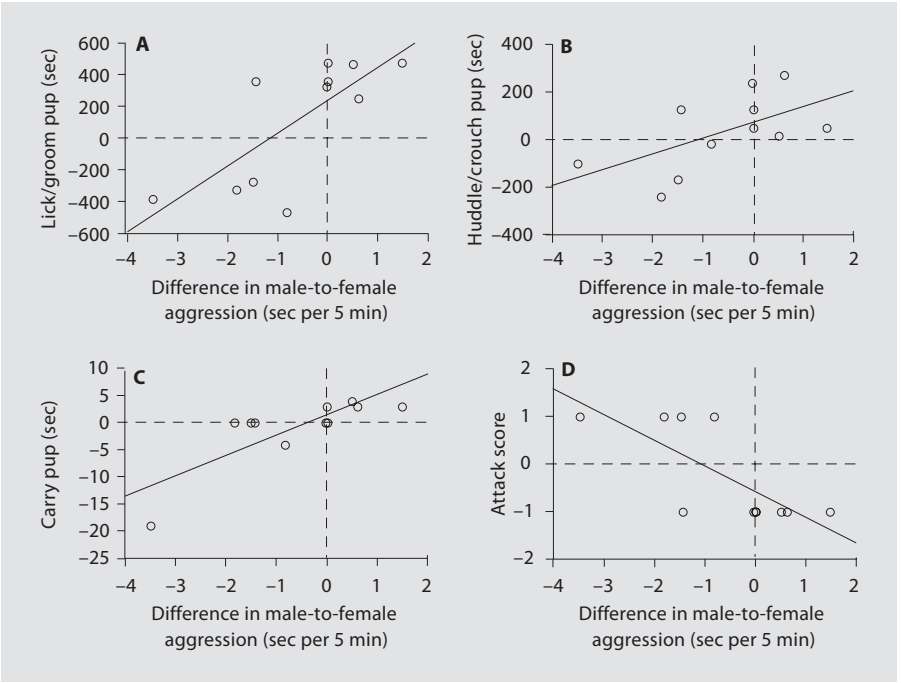

Fig. 4. Correlations comparing the difference between preferred and non-preferred males in the degree to which they directed aggression toward females and the difference in time males (**A**) licked or groomed pups, (**B**) huddled with or crouched over pups, (**C**) carried pups, or (**D**) their attack score. Dashed lines equal zero, where each male was equal for a given behavior. Positive values represent cases where preferred males engaged in more of a behavior than non-preferred males, whereas negative values represent the opposite.

(two-tailed binomial test: Preferred males; $n_{PAR} = 9$, $n_{NPAR} = 6$; $p = 0.61$).

Do Females Prefer Males with Greater V1aR Expression?

V1aR expression did not differ significantly in any of the five neural structures for preferred and non-preferred males (Student's t test: VP, $t_{(13)} = 0.49$, $p = 0.63$; LS, $t_{(13)} = 0.59$, $p = 0.56$; BST, $t_{(13)} = 0.20$, $p = 0.85$; MD Thal, $t_{(13)} = 0.14$, $p = 0.89$; LD Thal, $t_{(13)} = 1.11$, $p = 0.29$; fig. 5).

Does V1aR Expression Relate to Affiliative or Paternal Behavior?

The LD thalamic V1aR expression was negatively correlated with allogrooming during the choice test, however no other correlations were found between these structures and affiliation in either the paternal care test or the choice test (table 3). Therefore, V1aR expression in these areas does not appear to directly translate into affiliative behavior with infants or with potential mates.

Table 3. Correlation matrix for V1aR expression and either grooming behaviors from choice test or paternal care test

	Lick/groom pup (n = 13)	Allogrooming female (n = 15)
VP	r = 0.15; p = 0.62	r = –0.11; p = 0.71
LS	r = 0.09; p = 0.78	r = –0.12; p = 0.68
BST	r = 0.27; p = 0.38	r = –0.16; p = 0.58
MD Thal	r = 0.38; p = 0.21	r = –0.15; p = 0.61
LD Thal	r = 0.02; p = 0.95	r = –0.57; p = 0.03

V1aR expression (tissue equivalence in disintegrations per minute of ^{125}I-linear AVP ligand binding) was measured in the ventral pallidum (VP), lateral septum (LS), bed nucleus of the stria terminalis (BST), medial dorsal thalamus (MD Thal) and the lateral dorsal thalamus (LD Thal). We calculated the time spent allogrooming the females by averaging the proportion of time a given male allogroomed a female in each choice test in which he served. Sample sizes differed because some males did not survive long enough to harvest brains, or because of technical problems during behavioral tests. All p's were calculated using Fisher's r-to-*z* conversion.

Discussion

We initially expected females to attend to cues indicating good fathers and consistently choose those males. However, our data indicated that although females preferred affiliative males, affiliation did not predict which males were more paternal based on the proxy of paternal care we measured. Both preferred and non-preferred males were somewhat aggressive toward females, but mate choice was not associated with male aggression. In-

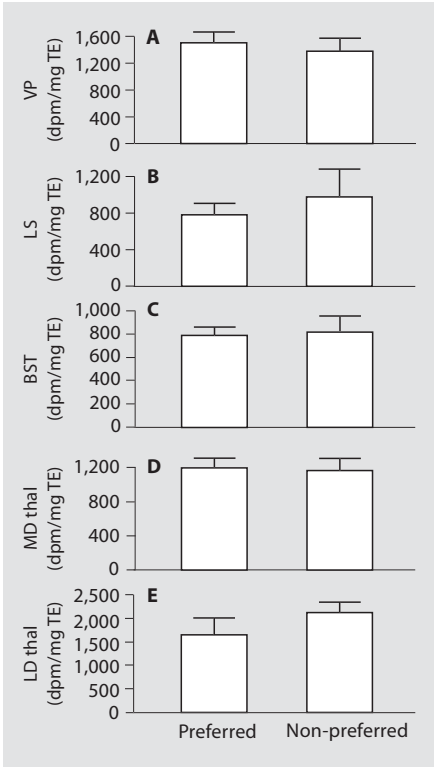

Fig. 5. Mean (± SE) tissue equivalence (TE) in disintegrations per minute (dpm) of radiolabeled V1aR autoradiographic ligand binding (^{125}I-linear AVP) in the (**A**) ventral pallidum, VP; (**B**) the lateral septum, LS; (**C**) the bed nucleus of the stria terminalis, BST; (**D**) the medal dorsal thalamus, MD Thal; and (**E**) the lateral dorsal thalamus, LD Thal for preferred and non-preferred males. n = 15, all p's > 0.05.

terestingly, male aggression was correlated with several aspects of paternal behavior. Lastly, almost none of the features of paternal behavior or mate choice we measured were correlated with the expression of male V1aR.

It was surprising that female-directed aggression can serve as an informative cue for parental males and that females appeared to ignore this cue. Why didn't females choose males based on their paternal behavior? Several possible explanations exist. First, our hypothesis assumes that paternal care increases female fitness. Paternal care has been shown to improve development of offspring in the laboratory [McGuire et al., 1992; Wang and Novak, 1992, 1994; Carter et al., 1995] but how laboratory results relate to a measure of fitness in the field is not clear. One field study in which fathers were experimentally removed when young were in the nest showed a decrease in juvenile recruitment (or survival to adulthood), likely resulting from infanticide by intruding strange males [Mahady and Wolff, 2002]. The most likely benefit male prairie voles provide in the field is defense against infanticide [Wolff and Macdonald, 2004], an important form of paternal care. Although territorial behavior and pup defense differ from the kind of pro-social paternal behavior we assessed, the behaviors could be collectively considered an adaptive suite of correlated paternal behaviors [Sih et al., 2004]. When viewed from this perspective, female-directed aggression might be a consequence of paternal behaviors, such as territorial defense, spilling over into the novel context of pairbond formation. Selection promoting territorial and paternal behavior might be the result of fitness benefits to males, rather than females [Reichard, 2003]. Other features of male prairie vole behavior (e.g., affiliation) or physiology (e.g., sperm production) [Ophir and delBarco-Trillo, 2007] might influence female fitness more directly or substantially than paternal behavior. In this case, these characteristics would take precedent over paternal abilities in mate-choice decisions. That females mated with 12 preferred and 4 non-preferred males, despite various levels of aggression by these individuals, attests to the fact that female-directed aggression was not a significant deterrent or attribute in mate choice. Although female-directed aggression may be related to, or at least predictive of, male paternal behavior, it may not be salient enough for females to base mate choices on this cue.

A second alternative is that our tests are imperfect proxies for paternal care. A male might treat his own offspring differently than he would alien pups. Although adult male prairie voles commonly perform alloparental behavior and readily accept novel pups [e.g., Wang and Novak, 1994; Carter et al., 1995], we do not know of any study that has directly tested the assumption that alloparental care predicts actual parental care in prairie voles.

Lastly, although our data indicate that female-directed aggression can serve as a cue for at least alloparental behavior, males might not have evolved signals of actual paternal care. This might be especially true if females are not equipped with the underlying mechanisms to detect any phenotypic expression of the genetic variance for paternal care [sensu Ryan et al., 1990]. In other words, the variance in amount of time licking, huddling, or grooming pups might not, in themselves, contribute directly to female fitness. In this case, such cues would not be relevant to female mating decisions and signals of parental expertise in males would not result from sexual selection. In any of these cases, the variance in paternal care associated with grooming, licking, and huddling with pups

might not be a sufficient criterion upon which sexual selection would operate.

For the most part, V1aR expression was not correlated with any of the behaviors we measured. It is possible that the experience of being either a preferred or non-preferred male could cause changes in V1aR expression or AVP release, which could potentially mask existing relationships between V1aR and behavior. However, Wang et al. [1997] showed that prairie vole V1aR expression is stable from birth to adulthood in the brain regions we examined. In addition, although sex differences in AVP are well documented in prairie voles [e.g., Wang et al., 1996], large samples of natural variation reveal no sex differences in V1aR [Phelps and Young, 2003]. Given that neither sex nor ontogeny seems to have an impact on prairie vole V1aR expression, it seems unlikely that behavioral changes in adult V1aR are confounding our analyses. AVP synthesis and release are rather dynamic, however, and could more readily interact with behavioral variation. For example, it is difficult to determine whether preferred males were more affiliative, or if an increase in affiliation is driven by female preference. In this scenario, affiliative behavior might have been elicited by the release of AVP due to the female's extended presence or cohabitation [Bamshad et al., 1994; Wang et al., 1994b]. In both, Bamshad et al. [1994] and Wang et al. [1994b], the animals were housed together for 3 days. We housed animals together for only 24 h and the time the animals actually interacted was even shorter (see table 1). If AVP release was responsible for the greater levels of affiliative behavior that we observed, then our data suggest that the effects of AVP on affiliation are faster than has been previously reported. Alternatively, males might exhibit individual variance in affiliative behaviors, which may then be amplified by subsequent AVP release. Nevertheless, if the behavioral differences we observed are directly related to underlying vasopressin machinery, it is likely that they relate to the more dynamic nature of AVP and its actions on V1aR than V1aR expression itself.

The concept of the 'endophenotype' emphasizes the fact that complex phenotypes can often be achieved through divergent mechanisms [Gottesman and Gould, 2003]; perhaps no phenotype is more complex than social behavior. Although preferred males were more affiliative with females, they did not show any differences in V1aR expression in areas associated with pairbonding or paternal behavior. It was also surprising that, with the exception of the LD Thal, V1aR expression in the neural areas we investigated did not relate to female or pup grooming directly despite its integral role in pairbonding and paternal care [Winslow et al., 1993; Wang et al., 1994a; Young and Wang, 2004]. When considering the dynamic nature of social interaction and the mechanistic complexity underlying these social behaviors [Young and Wang, 2004; Young et al., 2005], perhaps it is not surprising that clear patterns did not immediately emerge. Although vasopressin's action on V1aR does play an important role in pairbonding and paternal care, there are other proximate influences on how such social behaviors manifest themselves [e.g., oxytocin and dopamine, Young and Wang, 2004, and estrogen receptor alpha, Cushing and Wynne-Edwards, 2006]. Allelic diversity in the genetic architecture of the V1aR coding sequence may further complicate the story [Hammock and Young, 2005; Fink et al., 2006]. Our hypothesis that V1aR mediates correlated male traits subject to female choice was not supported by our data and suggests that a more elaborate explanation accounting for such complexity is required. Whether interactions between V1aR and other neuromodulatory mechanisms contribute to correlations among social behaviors remains to be demonstrated. Ultimately a better understanding of the complexity underlying these social behaviors will come from a better understanding of how these systems operate in concert.

Acknowledgments

We appreciate the helpful comments on earlier drafts of this manuscript from two anonymous reviewers. The research presented is described in and approved by Institutional Animal Care and Use Committee (IACUC) of the University of Florida, project number D289. This work was supported by funding from the National Science Foundation under grant numbers 0316631 and 0316451.

References

Bamshad M, Novak MA, Devries GJ (1994) Cohabitation alters vasopressin innervation and paternal behavior in prairie voles *(Microtus ochrogaster)*. Physiol Behav 56:751–758.

Carter CS, DeVries AC, Getz LL (1995) Physiological substrates of mammalian monogamy: The prairie vole model. Neurosci Biobehav Rev 19:303–314.

Clutton-Brock TH (1989) Mammalian mating systems. Proc R Soc Lond B Biol Sci 236:339–372.

Cushing BS, Wynne-Edwards KE (2006) Estrogen receptor-α distribution in male rodents is associated with social organization. J Comp Neurol 494:595–605.

Fink S, Excoffier L, Heckel G (2006) Mammalian monogamy is not controlled by a single gene. Proc Natl Acad Sci USA 103:10956–10960.
Gavish L, Carter CS, Getz LL (1981) Further evidence for monogamy in the prairie vole. Anim Behav 29:955–957.
Getz LL, McGuire B, Pizzuto T, Hofmann J, Frase B (1993) Social organization of the prairie vole *(Microtus ochrogaster)*. J Mammal 74: 44–58.
Goldizen AW (2003) Social monogamy in gibbons: The male perspective. In: Monogamy: Mating Strategies and Partnerships in Birds, Humans and Other Mammals (Reichard UH, Boesch C, eds), pp 232–247. Cambridge, UK: Cambridge University Press.
Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psych 160:636–645.
Gubernick DJ, Teferi T (2000) Adaptive significance of male parental care in a monogamous mammal. Proc R Soc Lond B Biol Sci 267:147–150.
Gubernick DJ, Wright SL, Brown RE (1993) The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. Anim Behav 46:539–546.
Hammock EAD, Young LJ (2005) Microsatellite instability generates diversity in brain and sociobehavioral traits. Science 308:1630–1634.
Hofmann JE, Getz LL (1988) Multiple exposures to adult males and reproductive activation of virgin female *Microtus ochrogaster*. Behav Proc 17:57–61.
Insel TR, Wang ZX, Ferris CF (1994) Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. J Neurosci 14:5381–5392.
Johnson AE, Audigier S, Rossi F, Jard S, Tribollet E, Barberis C (1993) Localization and characterization of vasopressin binding sites using an iodinated linear AVP antagonist. Brain Res 622:9–16.
Kleiman DG (1977) Monogamy in mammals. Q Rev Biol 52:39–69.
Lack D (1968) Ecological Adaptations for Breeding in Birds. London: Methuen.
Lim MM, Murphy AZ, Young LJ (2004) Ventral striato-pallidal oxytocin and vasopressin V1a receptors in the monogamous prairie vole *(Microtus ochrogaster)*. J Comp Neurol 468:555–570.
Liu Y, Curtis JT, Wang ZX (2001) Vasopressin in the lateral septum regulates pair bond formation in male prairie voles *(Microtus ochrogaster)*. Behav Neurosci 155:910–919.
Mahady S, Wolff JO (2002) A field test of the Bruce effect in the monogamous prairie vole, *Microtus ochrogaster*. Behav Ecol Sociobiol 52:31–37.
McGuire B, Drewsen Russell K, Mahoney T, Novak M (1992) The effects of mate removal on pregnancy success in prairie voles *(Microtus ochrogaster)* and meadow voles *(Microtus pennsylvanicus)*. Biol Rep 47:37–42.
Moehlman PD (1989) Intraspecific variation in canid social systems. In: Carnivore Behavior, Ecology, and Evolution (Gittleman JL, ed), pp 143–163. Ithaca, NY: Cornell University Press.
Ophir AG, delBarco-Trillo J (2007) Anogenital distance predicts female choice and male potency in prairie voles. Phys Behav, in press.
Orians GH (1969) On the evolution of mating systems in birds and mammals. Am Nat 103: 589–603.
Östlund S, Ahnesjö I (1998) Female 15-spined sticklebacks prefer better fathers. Anim Behav 56:1177–1183.
Pampoulie C, Lindström K, St. Mary CM (2004) Have your cake and eat it too: Male sand gobies show more parental care in the presence of female partners. Behav Ecol 15:199–204.
Petersen CW (1995) Male mating success and female choice in permanently territorial damselfishes. Bull Mar Sci 57:690–704.
Phelps SM, Young LJ (2003) Extraordinary diversity in vasopressin (V1a) receptor distributions among wild prairie voles *(Microtus ochrogaster)*: Patterns of variation and covariation. J Comp Neurol 466:564–576.
Pizzuto T, Getz LL (1998) Female prairie voles *(Microtus ochrogaster)* fail to form a new pair after loss of mate. Behav Process 43:79–86.
Reichard U (2003) Monogamy: Past and present. In: Monogamy: Mating Strategies and Partnerships in Birds, Humans and Other Mammals (Reichard UH, Boesch C, eds), pp 3–25. Cambridge, UK: Cambridge University Press.
Ryan MJ, Fox JH, Wilczynski W, Rand AS (1990) Sexual selection for sensory exploitation in the frog, *Physalaemus pustulosus*. Nature 343:66–68.
Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioral syndromes: An integrative overview. Q Rev Biol 79:241–277.
Thomas JA, Birney EC (1979) Parental care and mating system of the prairie vole, *Microtus ochrogaster*. Behav Ecol Sociobiol 5:171–186.
Thomas SA, Wolff JO (2004) Pair bonding and 'the widow effect' in female prairie voles. Behav Process 67:47–54
van Schaik KP, Kappeler PM (2003) The evolution of social monogamy in primates. In: Monogamy: Mating Strategies and Partnerships in Birds, Humans and Other Mammals (Reichard UH, Boesch C, eds), pp. 59–80. Cambridge, UK: Cambridge University Press.
Wang ZX, Novak MA (1992) The influence of the social environment on parental behavior and pup development of meadow voles *(Microtus pennsylvanicus)* and prairie voles *(Microtus ochrogaster)*. J Comp Psychol 106:163–171.
Wang ZX, Novak MA (1994) Alloparental care and the influence of the father's presence on juvenile prairie voles, *Microtus ochrogaster*. Anim Behav 47:281–288.
Wang Z, Ferris CF, DeVries GJ (1994a) Role of septal vasopressin innervation in paternal behavior in prairie voles *(Microtus ochrogaster)*. Proc Natl Acad Sci USA 91:400–404.
Wang ZX, Smith W, Major DE, De Vries GJ (1994b) Sex and species differences in the effects of cohabitation on vasopressin messenger RNA expression in the bed nucleus of the stria terminalis in prairie voles *(Microtus ochrogaster)* and meadow voles *(Microtus pennsylvanicus)*. Brain Res 650:212–218.
Wang Z, Young LJ, Liu Y, Insel TR (1997) Species differences in vasopressin receptor binding are evident early in development: Comparative anatomic studies in prairie montane voles. J Comp Neurol 378:535–546.
Wang Z, Zhou L, Hulihan TJ, Insel TR (1996) Immunoreactivity of central vasopressin and oxytocin pathways in Microtine rodents: A quantitative comparative study. J Comp Neurol 366:726–737.
Wiegmann DD, Baylis JR (1995) Male body size and paternal behaviour in smallmouth bass, *Micropterus dolomieui* (Pisces: Centrarchidae). Anim Behav 50:1543–1555.
Winslow JT, Hastings N, Cater CS, Harbaugh CR, Insel TR (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365:545–548.
Wittenberger JF, Tilson RL (1980) The evolution of monogamy: Hypotheses and evidence. Ann Rev Ecol Syst 11:197–232.
Wolff JO, Dunlap AS (2002) Multi-male mating, probability of conception, and litter size in the prairie vole *(Microtus ochrogaster)*. Behav Process 58:105–110.
Wolff JO, Macdonald DW (2004) Promiscuous females protect their offspring. Trends Ecol Evol 19:127–134
Young LJ, Wang Z (2004) The neurobiology of pair bonding. Nature Neurosci 7:1048–1054.
Young LJ, Lim MM, Grinrich B, Insel TR (2001) Cellular mechanisms of social attachment. Horm Behav 40:133–138.
Young LJ, Murphy Young AZ, Hammock EAD (2005) Anatomy and neurochemistry of the pair bond. J Comp Neurol 493:51–57.
Young LJ, Winslow JT, Nilsen R, Insel TR (1997) Species differences in V1a receptor gene expression in monogamous and nonmonogamous voles: Behavioral consequences. Behav Neurosci 111:599–605.